Novel Oleanolic Acid-Phtalimidines Tethered 1,2, 3 Triazole Hy-Brids As Promising Antibacterial Agents: Design, Synthesis, In Vitro Experiments and In Silico Docking Studies

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Article

Novel Oleanolic Acid-Phtalimidines Tethered 1,2,3 Triazole Hybrids as Promising Antibacterial Agents: Design, Synthesis, In Vitro Experiments and In Silico Docking Studies

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Abstract: As part of valorisation of agricultural waste into bioactive compounds, a series of structurally novel oleanolic acid ((3β-hydroxyolean-12-en-28-oic acid, **OA-1**)-phtalimidines (isoindolinones) conjugates **18a-v** bearing 1,2,3-triazole moieties were designed and synthesized by treating a previously azide **4** prepared from **OA-1** isolated from olive pomace (*Olea europaea* L.) with a wide range of propargylated phtalimidines using the Cu(I)-catalyzed click chemistry approach. **OA-1** and its newly prepared analogues **18a-v** were screened in vitro for their antibacterial activity against two Gram-positive bacteria, Staphylococcus aureus and *Listeria monocytogenes*; and two Gram-negative bacteria, *Salmonella thyphimurium* and *Pseudomonas aeruginosa*. Attractive results were obtained notably against *L. monocytogenes*. Compounds **18d**, **18g** and **18h** exhibited the highest antibacterial activity when compared with **OA-1** and other compounds in the series against tested pathogenic bacterial strains. Molecular docking study was performed to explore the binding mode of the most active derivatives against the target protein from *L. monocytogenes*. Results showed the importance of both hydrogen bonding and hydrophobic interactions with the target protein and are in favor to the experimental data.

Keywords: Oleanolic acid; phtalimidines; triazole; click chemistry; antibacterial activity; molecular docking

1. Introduction

Agricultural waste has emerged as a huge pool of fine chemicals that can be turned into high-value compounds with many pharmaceutical applications [1]. Triterpenic acids such as Oleanolic Acid (**OA-1**, Figure 1), extracted from olive pomace [2], is a typical example of such high-value compounds. A number of studies demonstrated that **OA-1** has a wide range of biological activities including anti- inflammatory [3], hepatoprotective [4], antioxidant [5], antitumor [6,7], anti-HIV [8], antidiabetic [9], and antiparasitic [10] effects. It has also found that **OA-1** and its derivatives exhibit

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appreciable antibacterial activities with a broad spectrum of MIC values [11–13]. The antibacterial activity of **OA-1** is thought to be due to its ability to influence peptidoglycan structure and composition, gene expression and biofilm formation, thereby preventing bacterial growth [14]. On the other hand, Nitrogen-containing heterocyclic compounds are omnipresent in bioactive molecules and are invaluable sources of therapeutic agents. Among them, Phtalimidine (1-isoindolinone and C3 1-isoindolinone-derived) derivatives [15] are playing an important role in drug discovery due to their broad and abundant biological activities [16–18].Many of them have been prepared and examined as antihypertensive [19], antipsychotic [20–22], anti-inflammatory [23], anesthetic [24], and vasodilatory [25] agents. Antiviral [26–29], anticancer [30–33], antimicrobial [34,35], and anxiolytic [36] activities have also been observed in this class of structures (Figure 1). Thus, enormous research has been taking place in the past few decades to synthesize and to explore various therapeutic prospective of this moiety [15,37,38].

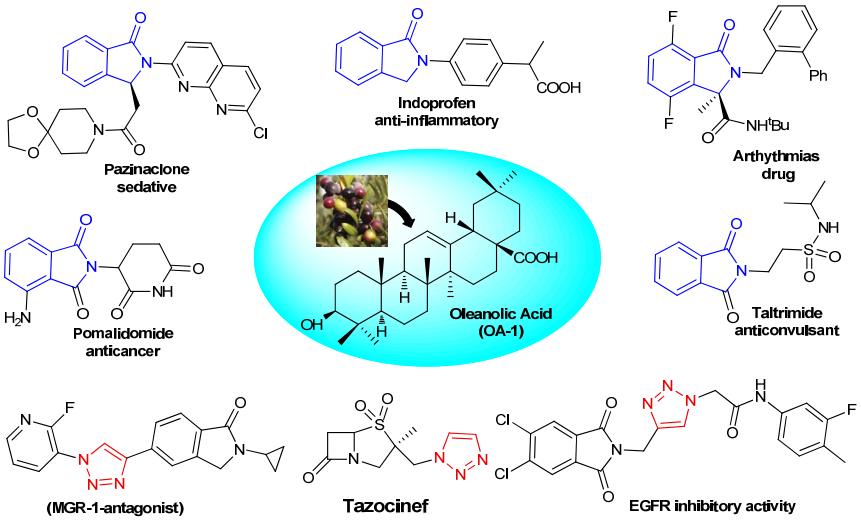

Figure 1. Oleanolic acid (**OA-1**) and representative biologically active molecules including Isoindolinone and 1,2,3-Triazole.

Despite the many potential uses of **OA-1**, it has not been developed into pharmaceuticals due to its instability and low water solubility. To overcome these drawbacks, several studies have been designed to modify the structure of **OA-1** with the hope of improving its physical properties for better bioavailability to enhance its bioactivity. Among the plethora of strategies used, molecular hybridization, which is a new concept in drug design and development based on the combination of two different bioactive compounds to produce a new hybrid substance, has emerged as an essential tool for design and construction of novel hybrid molecules with improved biological activities [39–45]. Considering the increasing incidence of multidrug resistant pathogens generated by extensive use of antibiotics [46], the interesting biological activities of isoindolinones and **OA-1**, our current research interest in the valorization of agricultural waste into eco-efficient, bioactive products, we are reporting herein the synthesis of novel triazole-tethered isoindolinones-oleanolic acid hybrids by means of click chemistry-mediated fusion between isoindolinones and oleanolic acid derivatives. To that end a Cu(I)-catalyzed azide alkyne Huisgen 1,3-cycloaddition was used [47–50]. Moreover, we hope that the introduction of triazole linkers, known by their broad and abundant biological properties (antiviral [51], antioxidant [52,53], antimicrobial [54], anticancer [55,56], antimalarial [57,58]...) could contribute to the improvement of the overall biological activity of the hybrid molecules. The antibacterial activity of **OA-1** and the target com-pounds was screened followed by in silico molecular docking studies for the most potent derivatives to get a distinct insight about the interactions and binding mode in the active sites of the target protein.

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2. Results and Discussion

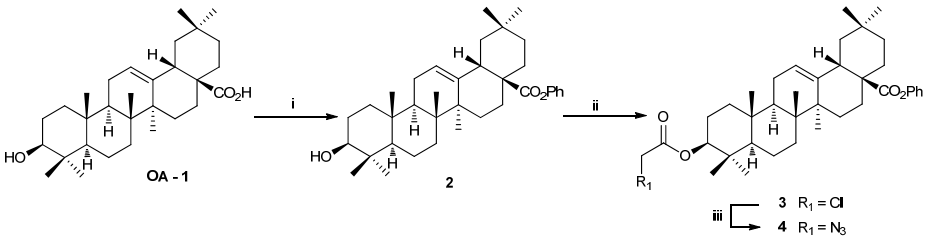
2.1. Chemistry

2.1.1. Isolation of oleanolic acid OA-1

OA-1 (Figure 1) was isolated from olive pomace (*Olea. europaea* L.) cultivar: Chemlali, by using a solid-liquid and ultrasound-assisted extraction strategy as previously described by us [59]. This method is low-cost, selective and providing a large amount of **OA-1** 6.8 g (3.4 mg/g DW)

2.1.2. Synthesis

The new hybrid molecules **18** were designed to include **OA-1** on one hand and isoindolinones **6**-**8** and (±)-**12**-**17** on the other, by connecting them via linker chains of different length. The Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) was chosen as the linking methodology. Starting from the naturally occurring triterpene **OA-1**, azidoacetyl **4** as first precursor was synthesized in three steps according a previously reported procedure (Scheme 1) [60,61].



Scheme 1. Synthesis of the azidoacetyl **4**. Reagents and Conditions: (i) K_2CO_3, BnBr, DMF, rt; (ii) $ClCH_2COCl$, $DMAP_{cat.}$, DCM, 0 °C; (iii) NaN_3, DMF, 75 °C.

The second precursors, namely N/O-propargylated isoindolinones **6**-**8** were also synthesized using previously published protocols [62–68]. Substrates **6a**-**g** were prepared through two synthetic routes involving base-assisted N-alkylation of phtalimide **5a**, hydantoin **5b**, succinimide **5c** and saccharin **5d** rings with propargyl bromide resulting in the isolation of the precursors **6a**-**d** in good isolated yields (87 to 97%). Under the same conditions, deprotonation then alkylation of N-hydroxyphthalimide **5g** afforded compound **6g** in 67% isolated yield. Applied to isoindolinone **5a** and successivelly but-3-yn-1-ol or but-3-yn-2-ol, mitsunobu reaction [69] (Ph_3P/DEAD/THF) provided isoindolinones **6e** and **6f** in 97% and 57% isolated yields, respectively (Scheme 2). Wishing to study the effect of the presence of a hydroxy and or acetoxy group on the antibacterial activity of our molecules, we subsequently prepared the hydroxylactams **7a** and **7e** as well as the acetoxylactam **8g**. In this sens, selective reduction of propargylated isoindolinones **6a** and **6e** was performed by using excess of $NaBH_4$ (4 equiv) in a dry MeOH at 0 °C [70] leading to the hydroxylactams **7a** and **7e** in 95% and 50% isolated yields, respectively (Scheme 2). Using our recently reported one-pot reduction–acetylation protocol, imide **6g** afforded the α-acetoxy-lactam **8g** in a 98% overall yield (Scheme 2) [71].

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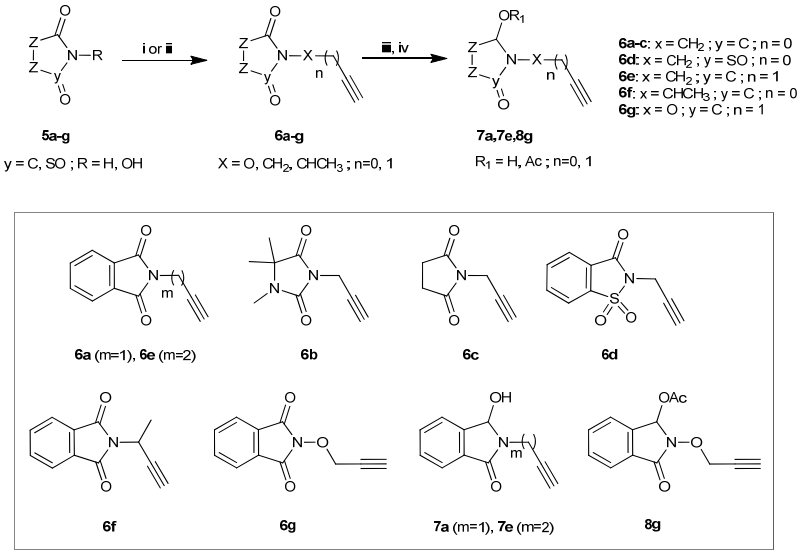

Scheme 2. Straightforward synthesis of substrates **6**-**8**. Reagents and Conditions for **6a-d** and **6g**: (i) imides (**5a-d,** R = H and **5g**, R = OH) (1 equiv), NaH or K_2CO_3 (1.1 equiv), propargyl bromide (1.2 equiv), DMF, reflux; Reagents and Conditions for **6e** and **6f**: (ii) imide (**5a**, R = H) (1 equiv), but-3-yn-1-ol or but-3-yn-2-ol (1.2 equiv), Ph_3P (1 equiv), $DEAD_{cat}$, THF, rt; Reagents and Conditions for **7a** and **7e**: iii) imides **6a** and **6e** (1 equiv), $NaBH_4$ (4 equiv), MeOH, 0–20 °C. Reagents and Conditions for **8g**: iv) a) **6g** (1 equiv), $NaBH_4$ (4 equiv), MeOH, 0–20 °C; b) Ac_2O (2 equiv), Et_3N (2 equiv), $DMAP_{cat}$, DCM, rt.

Subsequently, phtalimidines (±)-**12**-**17**, the other precursors for the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction, were obtained straightforwardly starting from homophthalic acid (**HPA**-**9**) as previously described by our group [72–74]. Phtalimidines (±)-**12a**-**f**, as starting materials, were accomplished by using our well-known 3-step sequence including: (i) esterification of diacid (**HPA**-**9**) under reflux in the presence of gaseous HCl, (ii) radical bromination of the obtained diester **10** (e.g., NBS, $AIBN_{cat}$, CCl_4 at reflux), and (iii) condensation of excess of primary amine (α-bromophthalate **11**, R-NH_2, CH_3CN, rt). Next, the C3-alkylated derivatives (±)-**13** and (±)-**14**, were prepared by the deprotonation of the α-position of the nitrogen of phtalimidines (±)-**12a**-**f** with potassium carbonate, followed by reaction of various halogenated electrophiles. Under these conditions, substrates (±)-**13a**-**d** and (±)-**14b**-**f**, were obtained in good to excellent isolated yields 87% to 90% for (±)-**13a**-**d** and 81% to 93% for (±)-**14b**-**f** (Scheme 3).

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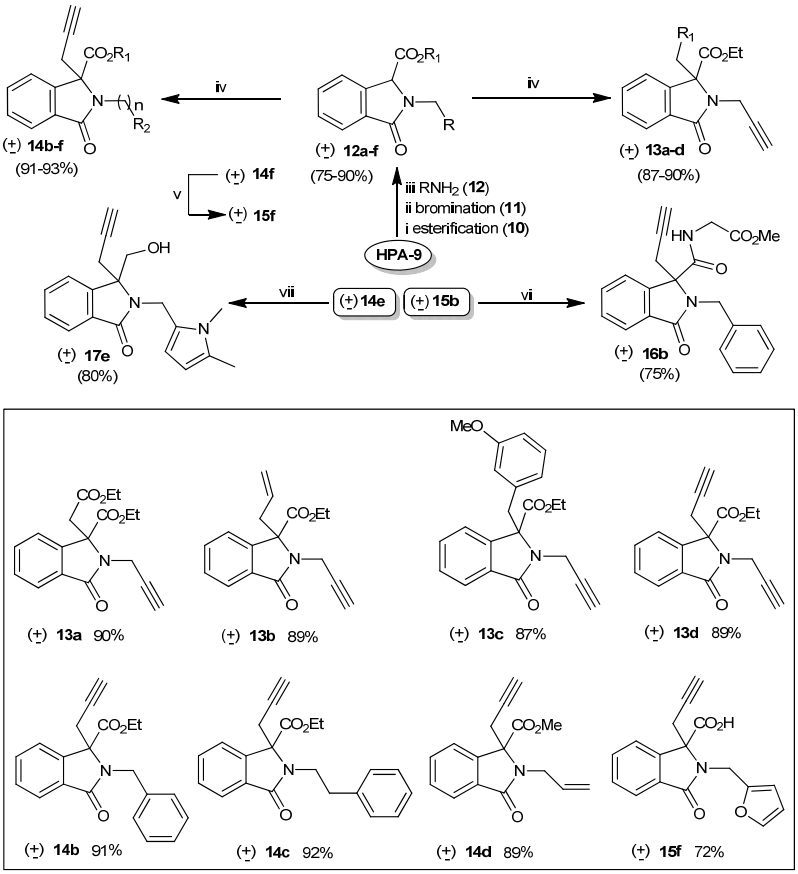

Scheme 3. Straightforward synthesis of substrates (±)-**12-17**. Reagents and Conditions for synthesis of phtalimidines (±)-**12**: (i) homophtalic acid **9**, gaseous HCl, reflux; (ii) ethyl homophtalate **10** (1 equiv), NBS (1.5 equiv), $AIBN_{cat}$, CCl_4, 60 °C. (iii) diethyl α-bromophthalate **11** (1 equiv), amine (2 equiv), CH_3CN, rt; Reagents and Conditions for synthesis of phtalimidines (±)-**13** and (±)-**14**:(iv) phtalimidines (±)-**12a-f** (1 equiv), K_2CO_3, alkylyl bromide (1.2 equiv), CH_3CN, reflux; Reagents and Conditions for (±)-**15f**: (v) phtalimidine (±)-**14f** (1 equiv), NaOH (2 equiv), $EtOH/H_2O$, rt then aqueous 1M HCl, 0 °C; Reagents and Conditions for (±)-**16b**: (vi) acid (±)-**15b** (1 equiv), EDCI (1 equiv), DMF, rt; Reagents and Conditions for (±)-**17e**: (vii) phtalimidine (±)-**14e** (1 equiv), $LiBH_4$ (2 equiv), DCM, rt.

In order to evaluate the binding mode, particulary the effect of an hydroxy, amide or carboxy group at C-3, on the antibacterial activity, acid (±)-**15f**, amide (±)-**16b** and alcohol (±)-**17e** were then prepared. Reduction of the ester group at C-3 of (±)-**14e** with $LiBH_4$ in dichloromethane at room temperature gave phtalimidine alcohol (±)-**17e** in 80% isolated yield. Saponification of ester functions of esters (±)-**14b** and (±)-**14f** under standars conditions (excess of aqueous NaOH, EtOH, rt then diluted HCl at 0 °C) gave the corresponding carboxylic acids (±)-**15b** and (±)-**15f** in respectively 93% and 72% isolated yields. In order to obtain amide (±)-**16b**, acid (±)-**15b** was treated, in a next step, with methyl glycinate in the presence of EDCI/DMF at room tempreture leading to compound (±)-**16b** in 75% isolated yield (Scheme 3) [75]. With azide **4** and propargylated phthalimidines **6-8** and (±)-**12-17** in hand, we next directed our attention to explore the 1,3-dipolar cycloaddition to form the new hybrid molecules **18**. Thus, as shown in Scheme 4, treatement of **4** and **6a** used as a model for our study under the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (e.g., $CuSO_4.5H_2O$, sodium ascorbate ($NaC_6H_7O_6$), DCM/H_2O, rt 24h) [76] resulted in the formation of the 1,2,3-triazole conjugate **18a** in 84% isolated yield.

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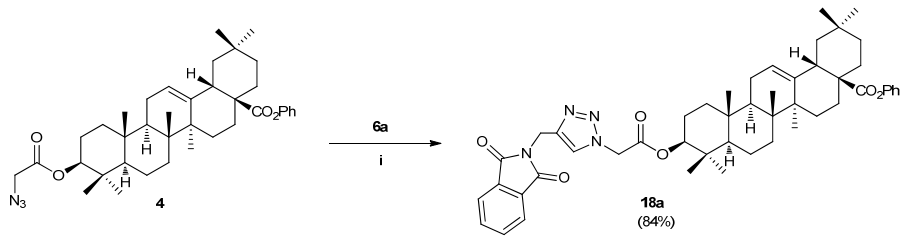
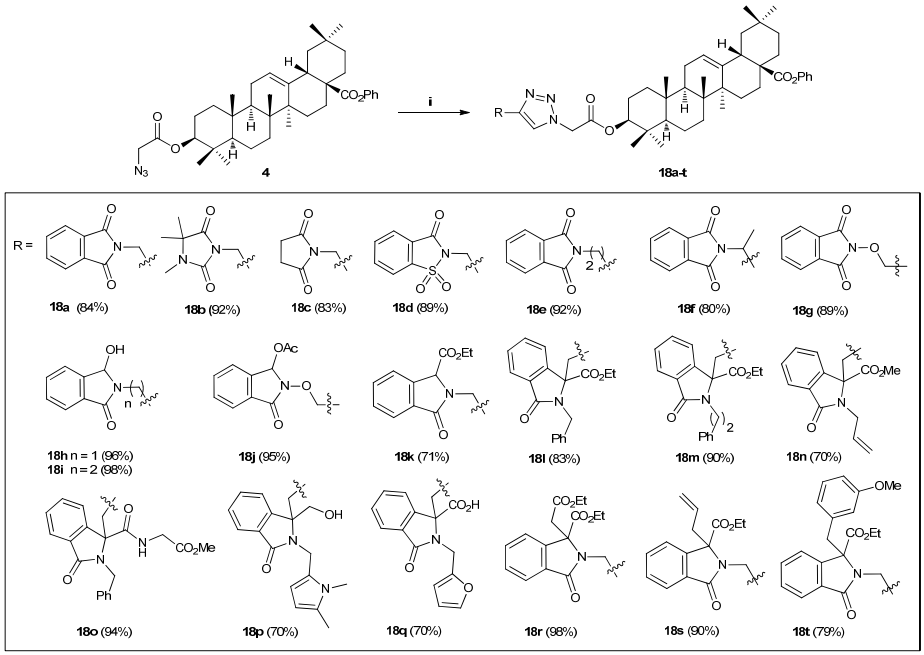


Scheme 4. Click synthesis of 1,2,3-triazole conjugate **18a**. Reagents and Conditions: (i) Phtalimidine **6a** (1 equiv), azide **4** (1 equiv), $CuSO_4{\cdot}5H_2O$ (0.2 equiv), sodium ascorbate (0.4 equiv), DCM/H_2O, rt, 24 h.

The above selected conditions were used with the rest of the substrates **6-8** and (±)-**12-17**, which cyclized to afford the hybrids molecules **18b-t** with yields of 70 to 98% after silica gel column chromatography, and the results are summarized in Scheme 5.



Scheme 5. Click synthesis of 1,2,3-triazole hybrids **18a-t**. Reagents and Conditions: (i) substrates **6-8**, (±)-**12-17** (1 equiv), azide **4** (1 equiv), $CuSO_4{\cdot}5H_2O$ (0.2 equiv), sodium ascorbate (0.4 equiv), DCM/H_2O, rt, 24 h.

It is worth noting that in the presence of the C,N-bispropargylated phtalimidine (±)-**13d** under standard conditions, the 1,3-dipolar cycloaddition became non selective and lead to a mixture of non separable products, including the mono-cycloaddition product on the C-propargylated alkyne, the mono-cycloaddition on the N-propargylated alkyne and the bis cycloadduct **18v**. After rigorous investigations, it was observed that the double cycloaddition occurred seamlessly to give **18v** in 80% yield when $CuSO_4.5H_2O$ (0.2 equiv), sodium ascorbate (0.4 equiv) and 2 equivalents of **4** were used (Scheme 6).

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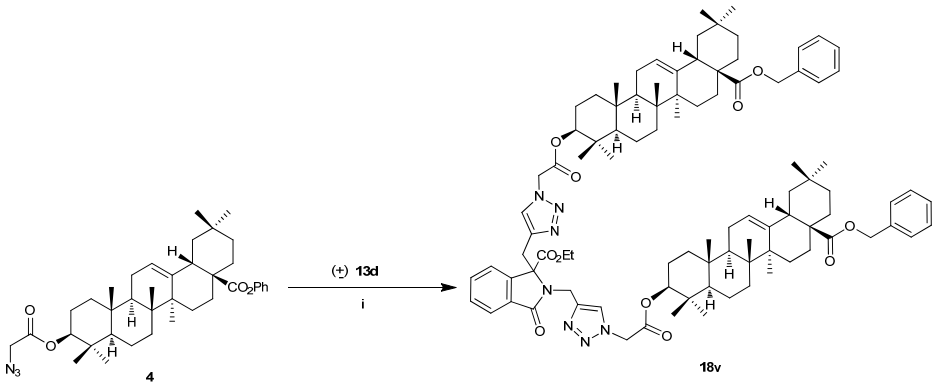

Scheme 6. Click synthesis of **18v**. Reagents and Conditions: (i) diproporgylated substrate (±)-**13d**, azide **4** (2 equiv), $CuSO_4{\cdot}5H_2O$ (0.2 equiv), sodium ascorbate (0.4 equiv), DCM/H_2O, rt, 24.

The structures of all the synthesized compounds **18** were confirmed by spectroscopic analysis. For example, the IR spectra of **18e**, shows a sharp intense band at 1714 cm^{-1} attributed to the four C=O moities; the ^1H NMR shows a singlet signal at δ_H 7.56 corresponding to the triazole proton and two triplets at δ_H 4.02 and 3.17 with coupling constant of 7.3 Hz corresponding to the two methylene groups at the junction of the phthalimide and the triazole moities. The ^{13}C NMR spectra, shows two characteristic signals at δ_C 177.5 and 168.2 corresponding to the C=O of the ester and the acetoxy groups, respectively, strong signal at δ_C = 166.1 ppm corresponding to the two C=O moieties of the Pht group was also showed.

2.2. Antibacterial avtivity

All the newly synthesized compounds were evaluated in vitro for their antibacterial activity against the following human pathogenstrains: two Gram-positive bacteria, Staphylococcus aureus ATCC 25923 and Listeria monocytogenes ATCC 19115; two Gram-negative bacteria, Salmonella thyphimurium ATCC 14080 and Pseudomonas aeruginosa ATCC 27853. The activity of all the tested derivatives was compared with that of Tetracycline used as standard reference antibiotic and the determination of the inhibition zone "IZ" (in mm), MIC and MBC (in µM) was carried out in this study. Overall, attractive results were obtained against certain strains. Indeed, the values of the IZ diameters, as a preliminary test, given in Table 1 for certain compounds were found to be in agreement with those of the MIC and MBC described in Table 2. These results showed that the starting product **OA-1** was active against S. aureus, S. thyphimurium and P. aeruginosa but inactive towards L. monocytogenes. The activity of this compound in several cases exceeds that of certain derivatives and of the reference antibiotic. It has been found to be more active than all its dervivatives **18a-v** against S. aureus and P. aeruginosa, but only more active than **18f**, **18k** and **18q** against S. typhimurium.The results showed, on the other hand, that most of the compounds tested proved to be quasi-selective towards L. monocytogenes. For more details, the most interesting results in terms of MIC were noted with the derivatives **18a**-**h** in addition to **18k**, **18m** and **18q** which showed good inhibitory effects on the growth of L. monocytogenes, compared to that of the reference antibiotic (MIC = 576.01 µM). Thus, from a structural point of view, the compound **18g** exhibits the highest activity (9.48 µM) towards this Gram-positive starin compared to the rest of the active compounds followed by derivatives **18d** (9.56 µM) and **18h** (9.89 µM). It appears that the 3-hydroxyisoindolin-1-one fragment in compound **18h** contributes to this activity compared to its analog **18a** with an isoindoline-1,3-dione moiety (MIC = 12.4 µM). This finding shows that the additional hydroxyl group in **18h** instead of the carbonyl function in **18a** may account for the noted difference in activity. On the other hand, the higher activity of compound **18a** against L. monocytogenes (Table 2), compared to that of its analog **18e** allows to conclude that a single methylene binding the phthalimide to the trizole was better than two methylenes giving, perhaps, to this system more free rotation and therefore less possibility of interaction with the target proteins of the bacterium. Also, we noticed that when the methylene linker directly bonded to the phthalimide nitrogen atom in **18g** (MIC = 39.01 µM and MBC

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= 2496.67 µM) is replaced by an oxygen atom in **18e** (MIC = 9.48 µM and MBC = 155.65 µM), the antibacterial potential towards L. monocytogenes was much improved, hence the importance of this new linker (-O-CH_2-) between phatlimide and triazole to better inhibit this strain. In addition, branching of the ethyl linker in **18e** was found to significantly improve the MBC of its analog **18f** (MBC = 624.16 µM). The additional methyl group in **18f** compared to **18a**, more than doubled the activity. Moreover, we noticed that compound **18a** with an isoindoline-1,3-dione moiety (MIC = 12.4 µM) remains more active against L. monocytogenes than its analogue **18c** with pyrrolidine-2,5-dione moiety (MIC = 12.4 µM). This finding serves as evidence that the antibacterial potential is affected by the additional aromatic ring.Interestingly, against P. aeruginosa, compound **18m** exhibited the highest antibacterial activity (MIC = 42.79 µM and MBC = 171.18 µM) compared to the other active compound **18h** (MIC = 158.41 µM and MBC = 633.66 µM) and also to the reference antibiotic. The results discussed above demonstrated the assumptions about the structural activity relationship (SAR) and also can be supported with some in silico studies.

Table 1. Antibacterial activity of **18a-v** expressed in Zone of Inhibition (mm).

Compound	*S. aureus* ATCC25923	*L. monocytogenes* ATCC19115	*S. thyphimurium* ATCC14080	*P. aeruginosa* ATCC27853
OA-1	12.2 ± 1.7^{a}	na	$12.8 \pm 1.1^{c,d}$	14.0 ± 1.0^{c}
18a	na	12.0 ± 1.4^{b}	na	na
18b	na	15.0 ± 0.0^{e}	na	na
18c	na	9.5 ± 2.1^{a}	na	na
18d	na	12.0 ± 0.0^{b}	na	na
18e	na	14.0 ± 0.0^{d}	na	na
18f	na	9.0 ± 0.0^{a}	12.0 ± 0.7^{c}	na
18g	na	15.0 ± 0.0^{e}	na	na
18h	na	12.0 ± 0.0^{b}	na	16.0 ± 0.0^{d}
18i	na	na	na	na
18j	na	na	na	na
18k	na	14.0 ± 0.7^{d}	10.0 ± 1.4^{a}	na
18l	na	na	na	na
18m	na	13.0 ± 0.0^{c}	na	13.0 ± 0.0^{b}
18n	na	na	na	na
18o	na	na	na	na
18p	na	na	na	na
18q	na	12.0 ± 0.7^{b}	11.0 ± 0.0^{b}	na
18r	na	na	na	na
18s	na	na	na	na
18t	na	na	na	na
18v	na	na	na	na
TET	18.0 ± 0.0^{b}	$12.5 \pm 0.0^{b,c}$	12.0 ± 0.1^{c}	11.0 ± 1.4^{a}

TET: tetracycline; na: not actif.

Table 2. Antibacterial activity of compounds **18a-v** expressed in MIC and MBC (µM).

Compound	*S. aureus* ATCC 25923		*L. monocytogenes* ATCC19115		*S. thyphimurium* ATCC14080		*P. aeruginosa* ATCC 27853	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
OA-1	78.02	171.18	147.9	1353.18	59.97	633.66	21.39	85.59
18a	na	na	12.4	1588.22	na	na	na	na
18b	na	na	59.97	1917.99	na	na	na	na
18c	na	na	21.13	1353.18	na	na	na	na
18d	na	na	9.56	151.86	na	na	na	na
18e	na	na	39.01	2496.67	na	na	na	na

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18f	na	na	39.01	624.16	78.02	312.08	na	na
18g	na	na	9.48	155.65	na	na	na	na
18h	na	na	9.89	633.66	na	na	158.41	633.66
18i	na	na	na	na	na	na	na	na
18j	na	na	na	na	na	na	na	na
18k	na	na	18.48	591.63	147.9	591.63	na	na
18l	na	na	na	na	na	na	na	na
18m	na	na	21.39	85.59	na	na	42.79	171.18
18n	na	na	na	na	na	na	na	na
18o	na	na	na	na	na	na	na	na
18p	na	na	na	na	na	na	na	na
18q	na	na	16.88	540.44	135.11	540.44	na	na
18r	na	na	na	na	na	na	na	na
18s	na	na	na	na	na	na	na	na
18t	na	na	na	na	na	na	na	na
18v	na	na	na	na	na	na	na	na
TET	4.5	288	576.01	1152.02	9	576.01	576.01	1152.02

TET: tetracycline; na: not actif.

2.3. Molecular docking study

The molecular docking simulations applied to antibacterial agents tested in vitro gives a descriptive explanation of the ligand's binding mode for the inhibition of the target bacterium [77,78]. In this context, to pick up the mode of action of the tested derivatives for their antibacterial potentials, the molecular docking study has been used to determine the binding modes against ABC substrate-binding protein Lmo0181 from *L. monocytogenes* (PDB ID: 5F7V). As depicted in Table 3, the listed binding affinities of the formed complex were found to be in the range of -12.3 to -10.6 kcal/mol. Thus, from these results, it can be suggested that all tested compounds interact favorably with the target protein and especially for derivatives **18c**, **18d**, **18h** and **18k** which showed the best binding scores (-12.3 to -11.6 kcal/mol) even better than the docked antibiotic "Tetracycline" (-11.1 kcal/mol) used as a reference in the in vitro test. Interestingly, as Figures 2 and 3 (A, B, C and D) show, the binding modes for these compounds demonstrate that each ligand was located inside the binding cavity, similar to the co-crystalized inhibitor. Therefore, the best docking score of compound **18h** could be attributed to its correct orientation in the receptor cavity (Figure 3: A) and it depends on its structure containing the 3-hydroxyisoindolin-1-one moiety, which could contribute to the stability of the receptor-ligand complex by the formation of three intermolecular conventional hydrogen bonds: two by its hydroxyl group with the residues Asn380 (2.55 Å) and Asp384 (2.12 Å), and another one by its carbonyl function with Thr75 (3.13 Å) amino acid (Figure 4: C'). In addition, the stability of the complex is also perceptible through other hydrophobic interactions formed via the hydrocarbon skeleton of the molecule: Alkyl, Pi-Alkyl and Pi-Pi as depicted in Table 4. The second-best score was designated to compound **18d** with -12.0 kcal/mol which is also involved in three conventional H-bonds formed through its benzo[d]isothiazol-3(2H)-one 1,1-dioxide fragment with Glu391 (3.27 Å) (3.38 Å) and its ester function with Trp271 (3.15 Å). As docking results of compound **18h**, the derivative **18d** displayed also some hydrophobic interactions (Figure 4: B'). On another hand, besides to other types of interactions, the ligand **18c** (-11.7 kcal/mol) showed a single hydrogen bond through the carbonyl function of its pyrrolidine-2,5-dione pharmacophore with Trp271 (3.13 Å) (Figure 4: A'), while the derivative **18k** (-11.6 kcal/mol) besides forming an hydrogen bond with Trp271 (3.25 Å), it displayed diverse interactions which additionally explain the stability of the docking complex as well as its inhibitory effect towards *L. monocytogenes* (Figure 4: D'). Attempt to validate QSAR model by using docking results demonstrated a high degree of correlation in terms of the ability to form hydrogen bonds, to display good binding scores and the antibacterial potentials of our synthesized compounds.

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Table 3. Binding energy of the docked compounds in the binding cavity of ABC substrate-binding protein Lmo0181 from *L. monocytogenes*.

Compound	Binding Energy (kcal/mol)
18a	-10.7
18b	-10.8
18c	-11.7
18d	-12.0
18e	-10.7
18f	-10.9
18g	-10.9
18h	-12.3
18k	-11.6
18m	-10.6
18q	-11.0
Tetracycline	-11.1

Table 4. Docking results of derivatives with lowest binding energy score and interacting residues the binding cavity of ABC substrate-binding protein Lmo0181 from L. monocytogenes.

Docked Compounds	Interacting Residues	Binding Energy (kcal/mol)
18c	van der Waals: Gln193, Val195, Thr196, Asn198, Leu249, Gln252, Leu272, Phe299, Glu391; H bond: Trp271*(3.13); Alkyl/Pi–Alkyl:Pro194(3.92)(4.90)(5.06), Pro246(5.14), Leu253(4.11)(5.26), Pro254(4.85)(5.21), Leu394(5.37) ; Pi-Pi: Phe70(4.10), Trp271(4.33).	-11.7
18d	van der Waals: Phe38, Asn71, Val148, Val195, Thr196, Asn198, Glu199, Pro246, Leu253, Thr268, Gly269, Asn301, Asn387, Leu394; H bond: Trp271*(3.15), Glu391**(3.27)(3.38); Alkyl/Pi–Alkyl: Phe70(5.14), Pro194(4.86), Pro254(4.77), Trp271(4.89), His297(4.07), Phe299 (4.43) (4.76); Pi-Pi: Phe70(3.87), Trp271(4.66) ; Pi-Anion: Glu391(3.81).	-12.0
18h	van der Waals: Phe38, Asn71, Asp72, Phe74, Thr196, Asn198, Glu199, Thr268, Gly269, Asn301, Glu338, Ser383, Asn387 ; H-bond: Thr75*(3.13), Asn*380(2.55), Asp384*(2.12); C-H bond: Thr75(3.28), Asp384(3.71)(3.76); Alkyl/Pi–Alkyl: Phe70(5.05)(5.26), Pro194(4.27)(5.19); Pi-Pi: Phe70(5.06), Trp271(4.47), Phe299(5.16).	-12.3
18k	van der Waals: Phe38, Asn71, Val148, Val195, Thr196, Asn198, Glu199, Pro246, Leu249, Gln252, Leu253, Thr268, Gly269, Asn301, Asn387 ; H-bond: Trp271*(3.25); Alkyl/Pi–Alkyl: Phe70(5.28), Trp271(4.89), Pro194(4.69)(4.89)(5.34) (5.36), Pro254(4.58), His297(3.99), Phe299(4.40)(4.72); Pi-Pi: Phe70(3.77), Trp271(4.45) ; Pi-Anion: Glu391(3.68).	-11.6
Tetracycline	van der Waals: Phe38, Ser39, Pro194, Asn198, Glu199, Leu253, Leu272, Asn301, Asn387; H-bond: Thr196*(2.12), Glu338*(2.82); Alkyl/Pi-Alkyl: Phe70(4.42), Trp271(5.47); Pi-Pi: Trp271(4.09)	-11.1

*: One H-bond; **: Two H-bonds.

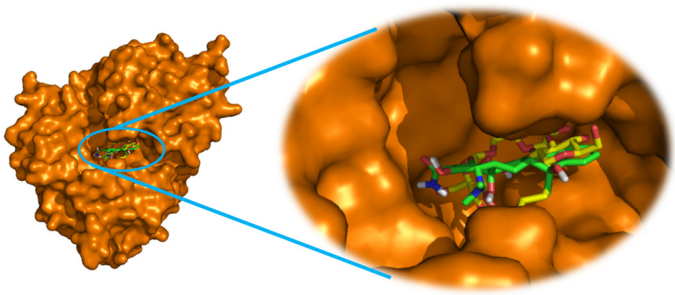

Figure 2. 3D binding modes of "Tetracycline" (green color) superimposed with the co-crystalized ligand "Cycloalternan" (yellow color) in the binding cavity of ABC substrate-binding protein Lmo0181 from *L. monocytogenes*.

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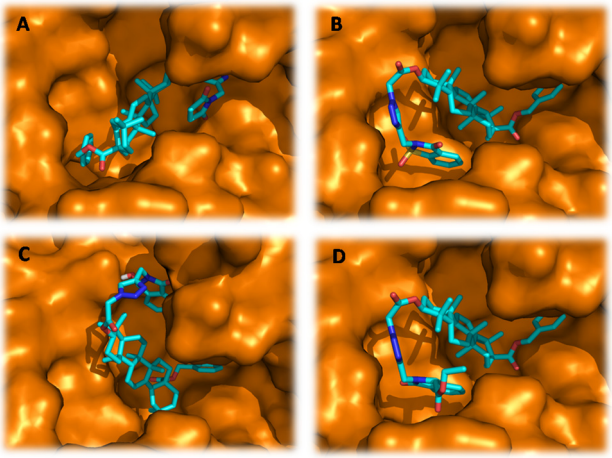

Figure 3. 3D binding modes of (**A**) co-crystalized ligand; (**B**) derivative **18c**; (**C**) derivative **18d**; (**D**) derivative**18h** and (**E**) derivative **18k** within the binding cavity of ABC substrate-binding protein Lmo0181 from *L. monocytogenes*.

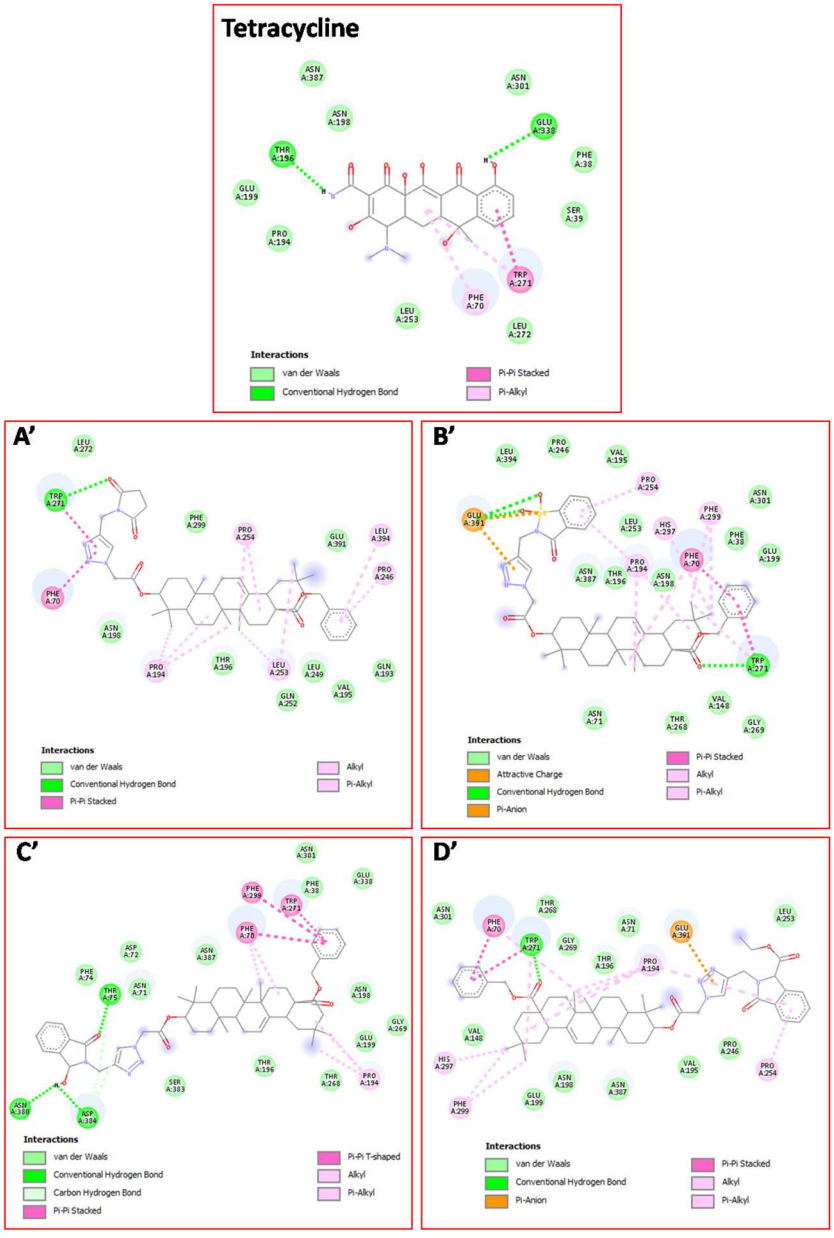

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Figure 4. 2D binding modes of "Tetracycline", (**A'**) derivative **18c**; (**B'**) derivative **18d**; (**C'**) derivative **18h** and (**D'**) derivative **18k** within the binding cavity of ABC substrate-binding protein Lmo0181 from *L. monocytogenes*.

3. Materials and Methods

3.1. General experimental procedure

Commercially available compounds were used without further purification (suppliers: Thermo Fisher Scientific Inc., and Sigma-Aldrich Co.). All glass apparatus was oven dried and cooled under vacuum before use. Before their usage, precautions were taken to eliminate moisture by refluxing over CaH_2 while distilling the solvents (CH_3CN, CH_2Cl_2). Column chromatographies were carried out with a BUCHI Pure Flash/Prep C-850 chromatography system using puriFlash® packed columns. Distilled solvents were employed as eluents for column chromatography in all cases. Thin layer chromatographies (TLC) was realized on sheets of silica gel 60 precoated with fluorescent indicator UV254 (Merck). Detection was performed by irradiation with a UV lamp and by using an ethanolic solution of *p*-anisaldehyde. Melting points were measured using a Stuart Scientific SMP10 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 1000 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AvanceIIITM 300 MHz spectrometer at room temperature (rt) with tetramethylsilane (TMS) serving as internal standard. Chemical shifts are expressed in parts per million (δ). Splitting patterns are designed: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; br s, broaden singlet. Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra (GC-MS) were obtained on a ThermoFinnigan Automass III spectrometer coupled with a gas chromatograph Trace GC 2000. An agilent 6530 Q-Tof MS system was used to conduct the measurment of high resolution mass spectra (HRMS).

3.2. Chemistry

3.2.1. General procedure for the preparation of compounds 18

To a mixture of equimolar amounts of azide 4 and propargylated phtalimidines **6**-**8** and (±)-**12**-**17** in CH_2Cl_2 (1 mL) and H_2O (1 mL) were added $CuSO_4.5H_2O$ (0.2 equiv.) and sodium ascorbate (0.4 equiv). After stirring at room temperature for 24h, the resulting residue was concentrated under vacuum then extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried using $MgSO_4$, filtered, and concentrated. The residue was then purified by flash column chromatography using a mixture of cyclohexane/EtOAc as eluent.

3.2.1.1. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-((1,3-dioxoisoindolin-2-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18a**)

This derivative was isolated as a white solid in 84% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 84-86 °C; $[\alpha]_D^{20}$ +57 (c 0.95 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2922.91 (CH str.), 1716.51 (4 x C=O); **^1H NMR (300 MHz, $CDCl_3$)** δ^H 7.90–7.83 (m, 2H$_{aro}$), 7.79–7.71 (m, 3H$_{aro}$), 7.41–7.30 (m, 5H$_{aro}$), 5.29 (t, *J* = 3.8 Hz, 1H), 5.09 (m, 6H), 4.54 (dd, *J* = 10.8, 5.2 Hz, 1H), 2.91 (dd, *J* = 14.2, 4.4 Hz, 1H) 1.12 (s, 3H), 0.92 (m, 6H), 0.85 (s, 3H), 0.79 (s, 3H), 0.64 (s, 3H), 0.60 (s, 3H); **^{13}C NMR (75 MHz, CDCl3)** δ^C 177.57 (C=O), 167.71 (C=O), 165.88 (2 x C=O), 143.88 (C^q), 136.57 (2 x C^q), 134.23 (2 x CH$_{aro}$), 132.19 (2 x C^q), 128.55 (2 x CH$_{aro}$), 128.10 (2 x CH$_{aro}$), 128.04 (CH$_{aro}$), 124.55 (CH$_{aro/trz}$), 123.59 (2 x CH$_{aro}$), 122.41 (CH), 83.97 (CH), 66.05 (CH$_2$), 55.27 (CH), 51.31 (CH$_2$), 47.61 (CH), 46.87 (C^q), 45.98 (CH$_2$), 41.80 (C^q), 41.50 (CH), 39.39 (C^q), 38.07 (CH$_2$), 37.79 (C^q), 36.95 (C^q), 33.98 (CH$_2$), 33.23 (CH$_3$), 33.09 (CH$_2$), 32.69 (CH$_2$), 32.49 (CH$_2$), 30.83 (C^q), 28.15 (CH$_3$), 27.73 (CH$_2$), 25.97 (CH$_3$), 23.78 (CH$_3$), 23.49 (2 x CH$_2$), 23.16 (CH$_2$), 18.22 (CH$_2$), 16.98 (CH$_3$), 16.54 (CH$_3$), 15.41 (CH$_3$); HRMS (+ESI) calculated for $C_{50}H_{63}N_4O_6$ [M+H$^+$]: 815.4703, found: 815.4775.

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3.2.1.2. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 2,2,6a,6b,9,9,12a-heptamethyl-10-(2-(4-((3,4,4-trimethyl-2,5-dioxoimidazolidin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18b**)

This derivative was isolated as a white solid in 92% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.4; mp = 92-94 °C; $[\alpha]_D^{20}$ +51 (c 1 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2923.14 (CH str.), 1770.86 (2 x C=O), 1711.07 (2 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.72 (s, 1H, $CH_{aro/trz}$), 7.38–7.28 (m, $5H_{aro}$), 5.27 (t, *J* = 3.5 Hz, 1H), 5.17–5.00 (m, 4H), 4.81 (s, 2H), 4.55 (dd, *J* = 9.9, 6.0 Hz, 1H), 2.94–2.83 (m, 4H), 1.36 (s, 6H), 1.10 (s, 3H), 0.93–0.78 (m, 12H) 0.71 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.45 (C=O), 176.03 (C=O), 165.81 (C=O), 154.68 (C=O), 143.75 (C^q), 143.01 (C^q), 136.43 (C^q), 128.42 (2 x CH_{aro}), 127.98 (2 x CH_{aro}), 127.92 (CH_{aro}), 124.38 ($CH_{aro/trz}$), 122.29 (CH), 83.79 (CH), 65.93 (CH_2), 61.39 (C^q), 55.17 (CH), 51.12 (CH_2), 47.49 (CH), 46.73 (C^q), 45.84 (CH_2), 41.68 (C^q), 41.36 (CH), 39.27 (C^q), 37.97 (CH_2), 37.73 (C^q), 36.85 (C^q), 33.80 (CH_2), 33.11 (CH_3), 32.57 (CH_2), 32.36 (CH_2), 30.71 (C^q), 29.71 (CH_2), 28.09 (CH_3), 27.59 (CH_2), 25.85 (CH_3), 24.41 (CH_3), 23.65 (CH_3), 23.38 (2 x CH_2), 23.03 (CH_2), 22.00 (2 x CH_3), 18.14 (CH_2), 16.85 (CH_3), 16.56 (CH_3), 15.31 (CH_3); HRMS (+ESI) calculated for $C_{48}H_{68}N_5O_6$ [M+H$^+$]: 810.5125, found: 810.5169.

3.2.1.3. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-((2,5-dioxopyrrolidin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18c**)

This derivative was isolated as a white solid in 83% yield; Rf (cyclohexane/EtOAc: 50/50) = 0.3; mp = 145-147 °C; $[\alpha]_D^{20}$ +108 (c 0.5 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2926.82 (CH str.), 1741.39 (C=O), 1721.47 (C=O), 1701.29 (2 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.71 (s, 1H, $CH_{aro/trz}$), 7.38–7.28 (m, $5H_{aro}$), 5.27 (t, *J* = 3.6 Hz, 1H), 5.17–4.99 (m, 4H), 4.80 (s, 2H), 4.54 (dd, *J* = 9.8, 6.1 Hz, 1H), 2.88 (dd, *J* = 13.6, 4.5 Hz, 1H), 2.71 (s, 4H), 1.10 (s, 3H), 0.93–0.85 (m, 9H), 0.81 (s, 3H), 0.71 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.51 (C=O), 176.54 (2 x C=O), 165.90 (C=O), 143.82 (C^q), 142.37 (C^q), 136.49 (C^q), 128.49 (2 x CH_{aro}), 128.06 (2 x CH_{aro}), 127.99 (CH_{aro}), 124.72 ($CH_{aro/trz}$), 122.35 (CH), 83.91 (CH), 66.00 (CH_2), 55.24 (CH), 51.16 (CH_2), 47.56 (CH), 46.79 (C^q), 45.91 (CH_2), 41.75 (C^q), 41.43 (CH), 39.33 (C^q), 38.03 (CH_2), 37.80 (C^q), 36.92 (C^q), 33.92 (CH_2), 33.68 (CH_2), 33.18 (CH_3), 32.63 (CH_2), 32.43 (CH_2), 30.78 (C_q), 28.30 (2 x CH_2), 28.16 (CH_3), 27.66 (CH_2), 25.93 (CH_3), 23.73 (CH_3), 23.46 (2 x CH_2), 23.10 (CH_2), 18.22 (CH_2), 16.92 (CH_3), 16.60 (CH_3), 15.40 (CH_3); HRMS (+ESI) calculated for $C_{46}H_{63}N_4O_6$ [M+H$^+$]: 767.4703, found: 767.4751.

3.2.1.4. (4aS,6aS,6bR,10S,12aR)-benzyl 10-(2-(4-((1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18d**)

This derivative was isolated as a white solid in 89% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 113-116 °C; $[\alpha]_D^{20}$ +80 (c 0.7 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2921.46 (CH str.),1725.07 (3 x C=O), 1181.72 (2 x S=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ^H 8.12–7.78 (m, $5H_{aro}$), 7.39–7.28 (m, $5H_{aro}$),5.27 (t, *J* = 3.69 Hz, 1H),5.21–4.97 (m, 6H), 4.53 (dd, *J* = 10.2, 5.6 Hz, 1H), 2.89 (dd, *J* = 13.7, 4.4 Hz, 1H), 1.10 (s, 3H), 0.93–0.87 (m, 6H), 0.83 (s, 3H), 0.78 (s, 3H), 0.65 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.56 (C=O), 165.77 (C=O), 158.59 (C=O),143.87 (C^q), 137.87 (C^q), 136.56 (2 x C^q), 135.09 (CH_{aro}), 134.56 (CH_{aro}), 128.54 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.03 (CH_{aro}), 127.31 (C^q), 125.48 (2 x CH_{aro}), 122.40 ($CH_{aro/trz}$), 121.24 (CH), 84.00 (CH), 66.04 (CH_2), 55.28 (CH), 47.59 (CH), 46.85 (C^q), 45.97 (CH_2), 41.79 (C^q), 41.49 (CH), 39.38 (C^q), 38.07 (CH_2), 37.80 (C^q), 36.94 (C^q), 33.98 (2 x CH_2), 33.21 (CH_3), 32.67 (CH_2), 32.47 (CH_2), 30.82 (C^q), 28.18 (CH_3), 27.72 (2 x CH_2), 25.96 (CH_3), 23.76 (CH_3), 23.48 (2 x CH_2), 23.15 (CH_2), 18.22 (CH_2), 16.97 (CH_3), 16.59 (CH_3), 15.38 (CH_3); HRMS (+ESI) calculated for $C_{49}H_{63}N_4O_7S$ [M+H$^+$]: 851.4373, found: 851.4464.

3.2.1.5. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-(2-(1,3-dioxoisoindolin-2-yl)ethyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18e**)

This derivative was isolated as a white solid in 92% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.4; mp = 94-96 °C; $[\alpha]_D^{20}$ +65 (c 0.85 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2929.05 (CH str.), 1714.31 (4 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.86–7.79 (m, $2H_{aro}$), 7.74–7.67 (m, $2H_{aro}$), 7.56 (s, 1H, $H_{aro/trz}$), 7.39–7.28

(m, 5H_{aro}), 5.27 (t, *J* = 3.6 Hz, 1H), 5.14–4.99 (m, 4H), 4.54 (dd, *J* = 10.0, 5.9 Hz, 1H), 4.02 (t, *J* = 7.3 Hz, 2H), 3.17 (t, *J* = 7.3 Hz, 2H), 2.89 (dd, *J* = 14.0, 4.5 Hz, 1H), 1.11 (s, 3H), 0.92–0.86 (m, 9H), 0.81 (s, 3H), 0.71 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,CDCl$_3$)** δ^C 177.55 (C=O), 168.28 (C=O), 166.13 (2 x C=O), 144.80 (C^q), 143.86 (C^q), 136.54 (C^q), 134.08 (2 x CH_{aro}), 132.17 (2 x C^q), 128.53 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.03 (CH_{aro}), 123.43 (2 x CH_{aro}), 122.88 ($CH_{aro/trz}$), 122.40 (CH), 83.84 (CH), 66.04 (CH_2), 55.30 (CH), 51.20 (CH_2), 47.60 (CH), 46.84 (C^q), 45.96 (CH_2), 41.79 (C^q), 41.48 (CH), 39.38 (C^q), 38.08 (CH_2), 37.84 (C^q), 37.45 (CH_2), 36.96 (C_q), 33.96 (CH_2), 33.21 (CH_3), 32.68 (CH_2), 32.47 (CH_2), 30.81 (C_q), 28.19 (CH_3), 27.71 (CH_2), 25.96 (CH_3), 24.96 (CH_2), 23.76 (CH_3), 23.49 (2 x CH_2), 23.14 (CH_2), 18.25 (CH_2), 16.96 (CH_3), 16.64 (CH_3), 15.43 (CH_3); HRMS (+ESI) calculated for $C_{51}H_{65}N_4O_6$ [M+H$^+$]: 829.4859, found: 829.4959.

3.2.1.6. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-(1-(1,3-dioxoisoindolin-2-yl)ethyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18f**)

This derivative was isolated as a white solid in 80% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 82-84 °C; [α]$_D^{20}$ +40 (*c* 1 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2942.80 (CH str.), 1776.94 (C=O), 17113.95 (3 x C=O); **^{1}H NMR (300 MHz, CDCl$_3$)** δ^H 7.84–7.77 (m, 3H_{aro}), 7.73–7.66 (m, 2H_{aro}), 7.38–7.28 (m, 5H_{aro}), 5.81 (q, *J* = 7.3 Hz, 1H), 5.27 (t, *J* = 3.6 Hz, 1H), 5.21–5.00 (m, 4H), 4.53 (dd, *J* = 10.5, 5.4 Hz, 1H), 2.89 (dd, *J* = 13.6, 4.4 Hz, 1H), 1.86 (d, *J* = 7.3 Hz, 3H), 1.10 (s, 3H), 0.93–0.87 (m, 6H), 0.87–0.82 (s, 3H), 0.81–0.75 (s, 3H), 0.70–0.61 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,CDCl$_3$)** δ^C 177.54 (C=O), 167.76 (C=O), 165.67 (2 x C=O), 147.82 (C^q), 143.85 (C^q), 136.53 (2 x C^q), 134.13 (2 x CH_{aro}), 132.05 (C^q), 128.53 (2 x CH_{aro}), 128.08 (2 x CH_{aro}), 128.02 (CH_{aro}), 123.84 ($CH_{aro/trz}$), 123.40 (2 x CH_{aro}), 122.39 (CH), 83.94 (CH), 66.03 (CH_2), 55.26 (CH), 51.32 (CH_2), 47.58 (CH), 46.83 (C^q), 45.94 (CH_2), 42.62 (CH), 41.77 (C^q), 41.46 (CH), 39.35 (C^q), 38.05 (CH_2), 37.78 (C^q), 36.93 (C^q), 33.95 (CH_2), 33.21 (CH_3), 32.65 (CH_2), 32.45 (CH_2), 30.81 (C^q), 28.15 (CH_3), 27.70 (CH_2), 25.95 (CH_3), 23.75 (CH_3), 23.46 (2 x CH_2), 23.13 (CH_2), 18.42 (CH_3), 18.21 (CH_2), 16.95 (CH_3), 16.58 (CH_3), 15.40 (CH_3); HRMS (+ESI) calculated for $C_{51}H_{65}N_4O_6$ [M+H$^+$]: 829.4859, found: 829.4942.

3.2.1.7. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-(((1,3-dioxoisoindolin-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18g**)

This derivative was isolated as a white solid in 89% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.4; mp = 107-109 °C; [α]$_D^{20}$ +77 (*c* 0.7 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2945.81 (CH str.), 1791.82 (C=O), 1729.79 (3 x C=O); **^{1}H NMR (300 MHz, CDCl$_3$)** δ^H 8.00 (s, 1$H_{aro/trz}$), 7.82–7.69 (m, 4H_{aro}), 7.38–7.28 (m, 5H_{aro}), 5.39 (s, 2H), 5.28 (t, *J* = 3.5 Hz, 1H), 5.17 (s, 2H), 5.12–4.99 (m, 2H), 4.58 (t, *J* = 7.9 Hz, 1H), 2.89 (dd, *J* = 13.9, 4.3 Hz, 1H), 1.11 (s, 3H), 0.94–0.82 (m, 12H), 0.75 (s, 3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz, CDCl$_3$)** δ^C 177.56 (C=O), 165.87 (C=O), 163.56 (2 x C=O), 143.86 (C^q), 142.13 (C^q), 136.54 (C^q), 134.61 (2 x CH_{aro}), 128.95 (2 x C^q), 128.53 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.03 (CH_{aro}), 126.09 ($CH_{aro/trz}$), 123.73 (2 x CH_{aro}), 122.39 (CH), 84.05 (CH), 70.52 (CH_2), 66.04 (CH_2), 55.30 (CH), 51.30 (CH_2), 47.60 (CH), 46.84 (C^q), 45.95 (CH_2), 41.79 (C^q), 41.47 (CH), 39.37 (C^q), 38.09 (CH_2), 37.87 (C^q), 36.96 (C^q), 33.96 (CH_2), 33.21 (CH_3), 32.67 (CH_2), 32.46 (CH_2), 30.82 (C^q), 28.22 (CH_3), 27.70 (CH_2), 25.96 (CH_3), 23.76 (CH_3), 23.51 (2 x CH_2), 23.14 (CH_2), 18.25 (CH_2), 16.96 (CH_3), 16.68 (CH_3), 15.44 (CH_3); HRMS (+ESI) calculated for $C_{50}H_{63}N_4O_7$ [M+H$^+$]: 831.4652, found: 831.4703.

3.2.1.8. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-((1-hydroxy-3-oxoisoindolin-2-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18h**)

This derivative was isolated as a white solid in 96% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.4; mp = 88-90 °C; [α]$_D^{20}$ +24 (*c* 1 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 3350 (OH), 2928.90 (CH str.), 1699.10 (3 x C=O); **^{1}H NMR (300 MHz, CDCl$_3$)** δ^H 7.77 (s, 1$H_{aro/trz}$), 7.70 (d, *J* = 7.4 Hz, 1H_{aro}), 7.61–7.40 (m, 3H_{aro}), 7.39–7.27 (m, 5H_{aro}), 5.92 (s, 1H), 5.25 (t, *J* = 3.8 Hz, 1H), 5.17–4.98 (m, 4H), 4.90 (d, *J* = 13.4 Hz, 2H), 4.70 (d, *J* = 15.4 Hz, 1H), 4.49 (dd, *J* = 10.5, 5.5 Hz, 1H), 2.89 (dd, *J* = 14.0, 4.4 Hz, 1H), 1.09 (s, 3H), 0.92–0.86 (m, 6H), 0.84–0.78 (s, 3H), 0.77–0.72 (s, 3H), 0.64–0.59 (s, 3H), 0.57 (s, 3H); **^{13}C NMR (75 MHz,CDCl$_3$)** δ^C 177.53 (C=O), 167.40 (C=O), 165.89 (C=O), 144.25 (C^q), 143.80 (C^q), 136.49 (C^q), 132.42

(C^q), 131.46 (C^q), 129.65 (2 x CH_{aro}), 128.50 (2 x CH_{aro}), 128.04 (2 x CH_{aro}), 127.99 (CH_{aro}), 123.60 (2 x CH_{aro}), 123.26 ($CH_{aro/trz}$), 122.35 (CH), 83.93 (CH), 81.59 (CH), 66.00 (CH_2), 55.19 (CH), 51.25 (CH_2), 47.53 (CH), 46.79 (C^q), 45.91 (CH_2), 41.73 (C^q), 41.42 (CH), 39.32 (C^q), 37.99 (CH_2), 37.73 (C^q), 36.87 (C^q), 34.49 (CH_2), 33.92 (CH_2), 33.18 (CH_3), 32.61 (CH_2), 32.42 (CH_2), 30.77 (C^q), 28.09 (CH_3), 27.66 (CH_2), 25.93 (CH_3), 23.73 (CH_3), 23.42 (2 x CH_2), 23.10 (CH_2), 18.15 (CH_2), 16.91 (CH_3), 16.51 (CH_3), 15.35 (CH_3); HRMS (+ESI) calculated for $C_{50}H_{64}N_4O_6Na$ [M+Na^+]: 839.4718, found: 839.4752.

3.2.1.9. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-(2-(1-hydroxy-3-oxoisoindolin-2-yl)ethyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18i**)

This derivative was isolated as a white solid in 98% yield; Rf (cyclohexane/EtOAc: 40/60) = 0.5; mp = 111-113 °C; $[\alpha]_D^{20}$ +40 (*c* 1 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 3350 (OH), 2931.46 (CH str.), 1729.50 (3 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.68 (d, *J* = 7.4 Hz, 1$H_{aro/trz}$), 7.59–7.40 (m, 4H_{aro}), 7.37–7.28 (m, 5H_{aro}), 5.83 (d, *J* = 6.6 Hz, 1H), 5.39 (br.s, 1H), 5.27 (t, *J* = 3.7 Hz), 5.14–4.98 (m, 4H), 4.52 (t, *J* = 8.0 Hz, 1H), 3.92 (t, *J* = 6.7 Hz, 2H), 3.17 (t, *J* = 6.8 Hz, 2H), 2.89 (dd, *J* = 13.8, 4.5 Hz, 1H), 1.11 (s, 3H), 0.92–0.85 (m, 9H), 0.81–0.77 (s, 3H), 0.73–0.69 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.57 (C=O), 167.70 (C=O), 166.06 (C=O), 144.23 (C^q), 143.87 (C^q), 136.52 (C^q), 132.26 (CH_{aro}), 131.67 (2 x C^q), 129.63 (CH_{aro}), 128.53 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.03 (CH_{aro}), 123.48 (2 x CH_{aro}), 123.20 ($CH_{aro/trz}$), 122.38 (CH), 84.10 (CH), 82.51 (CH), 66.04 (CH_2), 55.27 (CH), 51.39 (CH_2), 47.59 (CH), 46.83 (C^q), 45.95 (CH_2), 41.78 (C^q), 41.46 (CH), 39.36 (C^q), 38.93 (CH_2), 38.06 (CH_2), 37.84 (C^q), 36.94 (C^q), 33.95 (CH_2), 33.22 (CH_3), 32.65 (CH_2), 32.46 (CH_2), 30.82 (C^q), 28.20 (CH_3), 27.70 (CH_2), 25.97 (CH_3), 24.87 (CH_2), 23.76 (CH_3), 23.49 (2 x CH_2), 23.13 (CH_2), 18.24 (CH_2), 16.95 (CH_3), 16.65 (CH_3), 15.44 (CH_3); HRMS (+ESI) calculated for $C_{51}H_{67}N_4O_6$ [M+H^+]: 831.5016, found: 831.5037.

3.2.1.10. methyl2-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-oxoisoindoline-1-carboxylate (**18j**)

This derivative was isolated as a white solid in 95% yield; Rf (cyclohexane/EtOAc: 50/50) = 0.5; mp = 86-88 °C; $[\alpha]_D^{20}$ +52 (*c* 0.9 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2926.94 (CH str.), 1732.76 (4 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.96 (s, 1$H_{aro/trz}$), 7.79 (d, *J* = 7.8 Hz, 1H_{aro}), 7.64–7.43 (m, 3H_{aro}), 7.38–7.30 (m, 5H_{aro}), 7.03 (s, 1H), 5.33 (d, *J* = 1.7 Hz, 2H), 5.28 (t, *J* = 4.0 Hz, 1H), 5.17 (s, 2H), 5.13–4.99 (m, 2H), 4.57 (dd, *J* = 9.4, 6.6 Hz, 1H), 2.90 (dd, *J* = 14.0, 4.4 Hz, 1H), 2.17 (s, 3H), 1.11 (s, 3H), 0.93–0.85 (m, 9H), 0.83 (s, 3H), 0.73 (s, 3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.56 (C=O), 170.81 (C=O), 165.92 (C=O), 165.66 (C=O), 143.88 (C^q), 142.91 (C^q), 138.67 (C^q), 136.56 (C^q), 133.51 (CH_{aro}), 130.71 (CH_{aro}), 128.54 (2 x CH_{aro}), 128.10 (2 x CH_{aro}), 128.04 (CH_{aro}), 125.85 (CH_{aro}), 124.22 (CH_{aro}), 124.06 ($CH_{aro/trz}$), 122.41 (CH), 84.01 (CH), 80.82 (CH), 69.99 (C^q), 66.05 (CH_2), 55.31 (CH), 51.27 (CH_2), 47.61 (CH), 46.85 (C^q), 45.96 (CH_2), 41.80 (C^q), 41.49 (CH), 39.39 (C^q), 38.09 (CH_2), 37.87 (C^q), 36.97 (C^q), 33.98 (CH_2), 33.22 (CH_3), 32.69 (CH_2), 32.48 (CH_2), 30.83 (C^q), 29.83 (CH_2), 28.22 (CH_3), 27.72 (CH_2), 25.97 (CH_3), 23.77 (CH_3), 23.52 (2 x CH_2), 23.15 (CH_2), 21.18 (CH_3), 18.26 (CH_2), 16.97 (CH_3), 16.66 (CH_3), 15.45 (CH_3); HRMS (+ESI) calculated for $C_{52}H_{67}N_4O_8$ [M+H^+]: 875.4914, found: 875.4983.

3.2.1.11. ethyl2-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-oxoisoindoline-1-carboxylate (**18k**)

This derivative was isolated as a white solid in 71% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 91-93 °C; $[\alpha]_D^{20}$ +47 (*c* 0.95 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2943.22 (CH str.), 1702.18 (4 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.82 (d, *J* = 7.3 Hz, 1H_{aro}), 7.74 (s, 1$H_{aro/trz}$), 7.65–7.45 (m, 3H_{aro}),7.39–7.29 (m, 5H_{aro}), 5.43–5.34 (m, 1H), 5.31–5.23 (m, 2H), 5.14–5.00 (m, 4H),4.62–4.46 (m, 2H),4.40–4.20 (m, 2H), 2.91 (dd, *J* = 13.2, 4,1 Hz, 1H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.09 (s, 3H), 0.93–0.86 (m, 6H), 0.84–0.77 (s, 3H), 0.77–0.70 (s, 3H), 0.61–0.52 (m, 6H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.58 (C=O), 168.50 (C=O), 168.08 (C=O), 165.81 (C=O), 143.78 (2 x C^q), 139.72 (C^q), 135.56 (2 x C^q), 132.20 (CH_{aro}), 129.32 (CH_{aro}), 128.55 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.04 (CH_{aro}), 124.52 (CH_{aro}), 124.05 (CH_{aro}), 123.16 ($CH_{aro/trz}$), 122.40 (CH), 83.96 (CH), 66.05 (CH_2), 62.45 (CH_2), 62.01 (CH), 55.23 (CH), 51.29 (CH_2), 47.58 (CH), 46.85 (C^q), 45.95 (CH_2), 41.78 (C^q), 41.48 (CH), 39.37 (C^q), 38.03 (CH_2), 37.76 (C^q), 36.92 (C^q), 36.60 (CH_2),

33.98 (CH_2), 33.22 (CH_3), 32.66 (CH_2), 32.48 (CH_2), 30.83 (C^q), 28.13 (CH_3), 27.71 (CH_2), 25.96 (CH_3), 23.77 (CH_3), 23.47 (2 x CH_2), 23.14 (CH_2), 18.20 (CH_2), 16.97 (CH_3), 16.47 (CH_3), 15.40 (CH_3), 14.33 (CH_3); HRMS (+ESI) calculated for $C_{53}H_{69}N_4O_7$ [M+H$^+$]: 873.5122, found: 873.5161.

3.2.1.12. ethyl2-benzyl-1-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-oxoisoindoline-1-carboxylate (**18l**)

This derivative was isolated as a white solid in 83% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 105-106 °C; $[\alpha]_D^{20}$ +13 (*c* 0.7 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2927.79 (CH str.), 1710 (2 x C=O), 1700.90 (2 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.78 (d, *J* = 7.5 Hz, 1H$_{aro}$), 7.60–7.39 (m, 5H$_{aro}$), 7.36–7.27 (m, 6H$_{aro}$), 7.25–7.20 (m, 1H$_{aro}$), 6.18 (d, *J* = 9.4 Hz, 1H$_{aro}$), 5.27 (t, *J* = 3.7 Hz, 1H), 4.94–4.62 (m, 4H), 4.53–4.43 (m, 1H), 3.90–3.74 (m, 3H), 2.89 (dd, *J* = 13.6, 4.4 Hz, 1H), 1.25 (t, *J* = 7.5 Hz, 3H), 1.10 (s, 3H), 0.93–0.87 (m, 9H), 0.80–0.72 (s, 3H), 0.71–0.64 (s, 3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.56 (C=O), 169.99 (C=O), 169.38 (C=O), 165.84 (C=O), 143.87 (C^q), 143.76 (C^q), 140.77 (C^q), 137.17 (C^q), 136.54 (C^q), 132.35 (CH_{aro}), 129.37 (3 x CH_{aro}) , 128.53 (4 x CH_{aro}), 128.11 (2 x CH_{aro}), 128.04 (CH_{aro}), 127.69 (CH_{aro}), 124.06 (CH_{aro}), 123.08 ($CH_{aro/trz}$), 122.41 (CH), 122.00 (CH_{aro}), 83.72 (CH), 70.76 (C^q), 66.05 (CH_2), 62.33 (CH_2), 55.28 (CH), 50.94 (CH_2), 47.61 (CH), 46.84 (C^q), 45.96 (CH_2), 44.87 (CH_2), 41.79 (C^q), 41.47 (CH), 39.38 (C^q), 38.08 (CH_2), 37.86 (C^q), 37.82 (C^q), 36.95 (C^q), 33.97 (CH_2), 33.22 (CH_3), 32.68 (CH_2), 32.47 (CH_2), 30.82 (C^q), 29.85 (CH_2), 28.18 (CH_3), 27.71 (CH_2), 25.96 (CH_3), 23.76 (CH_3), 23.48 (2 x CH_2), 23.15 (CH_2), 18.26 (CH_2), 16.96 (CH_3), 16.65 (CH_3), 15.43 (CH_3), 13.61 (CH_3); HRMS (+ESI) calculated for $C_{60}H_{75}N_4O_7$ [M+H$^+$]: 963.5591, found: 963.5641.

3.2.1.13. ethyl1-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-oxo-2-phenethylisoindoline-1-carboxylate (**18m**)

This derivative was isolated as a white solid in 90% yield; Rf (cyclohexane/EtOAc: 70/30) = 0.5; mp = 93-95 °C; $[\alpha]_D^{20}$ +73 (*c* 0.75 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2936.05 (CH str.), 1708.51 (4 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.77 (d, *J* = 7.4 Hz, 1H$_{aro}$), 7.62–7.54 (m, 2H$_{aro}$), 7.53–7.45 (m, 1H$_{aro}$),7.40–7.27 (m, 9H$_{aro}$), 7.25–7.18 (m, 1H$_{aro}$), 6.53 (d, *J* = 18.8 Hz, 1H$_{aro}$), 5.27 (t, *J* = 3.7 Hz, 1H), 5.12–5.01 (m, 2H), 4.88 (qd, *J* = 17.43, 17.43, 17.42, 2.38 Hz, 2H), 4.52–4.43 (m, 1H), 4.28–4.10 (m, 2H), 4.03–3.93 (m, 1H), 3.87–3.77 (m, 1H), 3.67 (t, *J* = 7.9 Hz, 2H), 3.18–2.98 (m, 2H), 2.90 (dd, *J* = 13.7, 4.5 Hz, 1H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.10 (s, 3H), 0.93–0.84 (m, 9H), 0.76 (s, 3H), 0.68 (s, 3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.55 (C=O), 170.31 (C=O), 169.22 (C=O), 165.83 (C=O), 143.86 (C^q), 143.28 (C^q), 140.92 (C^q), 139.24 (C^q), 136.54 (C^q), 132.29 (CH_{aro}), 129.61 (CH_{aro}), 129.05 (2 x CH_{aro}), 128.68 (2 x CH_{aro}), 128.54 (2 x CH_{aro}), 128.11 (2 x CH_{aro}), 128.04 (CH_{aro}), 126.54 (CH_{aro}), 123.81 (CH_{aro}), 122.84 ($CH_{aro/trz}$), 122.41 (CH), 122.24 (CH_{aro}), 83.79 (CH), 71.45 (C^q), 66.05 (CH_2), 62.77 (CH_2), 55.27 (CH), 51.06 (CH_2), 47.60 (CH), 46.85 (C^q), 45.96 (CH_2), 44.25 (CH_2), 41.79 (C^q), 41.48 (CH), 39.37 (C^q), 38.06 (CH_2), 37.83 (C^q), 37.81 (C^q), 36.94 (C^q), 34.36 (CH_2), 33.97 (CH_2), 33.22 (CH_3), 32.68 (CH_2), 32.47 (CH_2), 30.82 (C^q), 30.69 (CH_2), 28.17 (CH_3), 27.71 (CH_2), 25.96 (CH_3), 23.77 (CH_3), 23.48 (2 x CH_2), 23.15 (CH_2), 18.25 (CH_2), 16.96 (CH_3), 16.65 (CH_3), 15.39 (CH_3), 14.09 (CH_3); HRMS (+ESI) calculated for $C_{61}H_{77}N_4O_7$ [M+H$^+$]: 977.5748, found: 977.5807.

3.2.1.14. methyl2-allyl-1-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-oxoisoindoline-1-carboxylate (**18n**)

This derivative was isolated as a white solid in 70% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.4; mp = 98-100 °C; $[\alpha]_D^{20}$ +80 (*c* 0.7 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2935.43 (CH str.), 1710.09 (4 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.75 (d, *J* = 7.4, 1.3 Hz, 1H$_{aro}$), 7.61–7.43 (m, 3H$_{aro}$), 7.37–7.29 (m, 5H$_{aro}$), 6.55 (d, *J* = 21.3 Hz, 1H$_{aro}$), 5.98–5.81 (m, 1H), 5.36–5.16 (m, 3H), 5.14–4.99 (m, 2H), 4.98–4.77 (m, 2H), 4.53–4.35 (m, 2H), 4.11–4.00 (m, 1H),3.88 (qd, *J* = 15.72, 15.71, 15.71, 4.49 Hz, 2H), 3.65 (s, 3H), 2.89 (dd, *J* = 13.5, 4.3 Hz, 1H), 1.10 (s, 3H), 0.94–0.82 (m, 9H), 0.77 (s, 3H), 0.73–0.65 (m, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.56 (C=O), 170.80 (C=O), 168.70 (C=O), 165.87 (C=O), 143.86 (2 x C^q), 140.86 (C^q), 136.53 (C^q), 133.11 (CH_{aro}), 132.35 (CH_{aro}), 129.61 (CH_{aro}), 128.53 (2 x CH_{aro}), 128.10 (2 x

CH_{aro}), 128.04 (CH_{aro}), 124.03 (CH_{aro}), 122.91 ($CH_{aro/trz}$), 122.40 (CH), 121.93 (CH) 118.54 (CH_2), 83.80 (CH), 70.35 (C^q), 66.04 (CH_2), 55.28 (CH), 53.10 (CH_3), 51.04 (CH_2), 47.60 (CH), 46.84 (C^q), 45.95 (CH_2), 44.15 (CH_2), 41.78 (C^q), 41.47 (CH), 39.37 (C^q), 38.07 (CH_2), 37.86 (C^q), 37.82 (C^q), 36.94 (C^q), 33.96 (CH_2), 33.22 (CH_3), 32.68 (CH_2), 32.46 (CH_2), 30.82 (C^q), 30.02 (CH_2), 28.16 (CH_3), 27.70 (CH_2), 25.95 (CH_3), 23.76 (CH_3), 23.47 (2 x CH_2), 23.14 (CH_2), 18.25 (CH_2), 16.95 (CH_3), 16.64 (CH_3), 15.42 (CH_3); HRMS (+ESI) calculated for $C_{55}H_{71}N_4O_7$ [M+H$^+$]: 899.5278, found: 899.5327.

3.2.1.15. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-((2-benzyl-1-((2-methoxy-2-oxoethyl)carbamoyl)-3-oxoisoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18o**)

This derivative was isolated as a white solid in 94% yield; Rf (cyclohexane/EtOAc: 40/60) = 0.5; mp = 120-122 °C; $[\alpha]_D^{20}$ +41 (*c* 1 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 3300 (NH), 2931.02 (CH str.), 1726.01 (3 x C=O), 1677.77 (C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.72 (d, *J* = 7.4 Hz, 1H_{aro}), 7.63–7.39 (m, 6H_{aro}), 7.36–7.27 (m, 6H_{aro}), 7.25–7.19 (m, 1H_{aro}), 6.32 (d, *J* = 11.7 Hz, 1H_{aro}), 5.86 (br.s, 1H), 5.27 (t, *J* = 3.8 Hz, 1H), 5.12–4.76 (m, 5H), 4.65 (dd, *J* = 15.2, 6.7 Hz, 1H), 4.01–3.91 (m, 1H), 3.96 (d, *J* = 15.6 Hz, 1H), 3.85–3.56 (m, 5H),3.28–3.14 (m, 1H), 2.89 (dd, *J* = 13.8, 4.4 Hz, 1H), 1.10 (s, 3H), 0.92–0.87 (m, 9H), 0.78 (s, 3H), 0.72–0.67 (s, 3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.55 (C=O), 169.83 (C=O), 169.18 (2 x C=O), 165.83 (C=O), 144.31 (C^q), 143.85 (C^q), 141.05 (C^q), 137.54 (C^q), 136.52 (C^q), 132.81 (CH_{aro}), 130.95 (C^q), 129.66 (CH_{aro}), 129.42 (2 x CH_{aro}), 128.83 (2 x CH_{aro}), 128.52 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.02 (CH_{aro}), 127.83 (CH_{aro}), 124.19 (CH_{aro}), 123.54 (CH_{aro}), 122.63 ($CH_{aro/trz}$), 122.39 (CH), 83.73 (CH), 71.66 (C^q), 66.04 (CH_2), 55.27 (CH), 52.34 (CH_3), 50.98 (CH_2), 47.60 (CH), 46.83 (C^q), 45.94 (CH_2), 44.84 (CH_2), 41.78 (C^q), 41.46 (CH), 41.29 (CH_2), 39.36 (C^q), 38.07 (CH_2), 37.86 (C^q), 36.94 (C^q), 33.95 (CH_2), 33.20 (CH_3), 32.67 (CH_2), 32.46 (CH_2), 30.81 (C^q), 29.81 (CH_2), 28.16 (CH_3), 27.69 (CH_2), 25.95 (CH_3), 23.75 (CH_3), 23.47 (2 x CH_2), 23.13 (CH_2), 18.24 (CH_2), 16.95 (CH_3), 16.64 (CH_3), 15.41 (CH_3); HRMS (+ESI) calculated for $C_{61}H_{75}N_5O_8Na$ [M+Na$^+$]: 1028.5508, found: 1028.5584.

3.2.1.16. (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-benzyl 10-(2-(4-((2-((1,5-dimethyl-1H-pyrrol-2-yl)methyl)-1-(hydroxymethyl)-3-oxoisoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetoxy)-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylate (**18p**)

This derivative was isolated as a yellow solid in 70% yield; Rf (cyclohexane/EtOAc: 40/60) = 0.5; mp = 85-87 °C; $[\alpha]_D^{20}$ +64 (*c* 0.85 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 3400 (OH), 2923.75 (CH str.), 1726.83 (C=O), 1678.10 (2 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.78 (d, *J* = 7.5 Hz, 1H_{aro}), 7.63–7.40 (m, 3H_{aro}), 7.39–7.28 (m, 6H_{aro}), 6.44 (d, *J* = 16.9 Hz, 1H_{aro}), 6.16 (t, *J* = 2.6 Hz, 1H), 5.84 (d, *J* = 3.3 Hz, 1H_{aro}), 5.27 (t, *J* = 3.7 Hz, 1H), 5.19–4.79 (m, 5H), 4.64–4.45 (m, 2H), 3.82–3.70 (m, 2H), 3.53–3.36 (m, 5H), 2.89 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.18 (s, 3H), 1.11 (s, 3H), 0.93–0.86 (m, 9H), 0.79 (s, 3H), 0.74–0.68 (s,3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.55 (C=O), 168.51 (C=O), 165.93 (C=O), 146.14 (C^q), 143.86 (C^q), 141.70 (C^q), 136.53 (C^q), 132.29 (C^q), 132.10 (CH_{aro}), 130.78 (C^q), 128.90 (CH_{aro}), 128.52 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.03 (CH_{aro}), 127.19 (C^q), 123.92 (CH_{aro}), 123.09 ($CH_{aro/trz}$), 122.39 (CH), 121.92 (CH_{aro}), 108.56 (CH_{aro}), 105.93 (CH_{aro}), 83.83 (CH), 70.12 (C^q), 66.04 (CH_2), 55.29 (CH), 51.05 (CH_2), 47.60 (CH), 46.84 (C^q), 45.96 (CH_2), 41.79 (C^q), 41.47 (CH), 39.38 (C^q), 38.08 (CH_2), 37.87 (C^q), 36.95 (C^q), 35.98 (CH_2), 33.96 (CH_2), 33.21 (CH_3), 32.68 (CH_2), 32.46 (CH_2), 30.88 (CH_3), 30.81 (C^q), 29.82 (CH_2), 29.48 (CH_2), 28.19 (CH_3), 27.71 (CH_2), 25.96 (CH_3), 23.75 (CH_3), 23.49 (2 x CH_2), 23.14 (CH_2), 18.26 (CH_2), 16.96 (CH_3), 16.67 (CH_3), 15.44 (CH_3), 12.66 (CH_3); HRMS (+ESI) calculated for $C_{58}H_{75}N_5O_6Na$ [M+Na$^+$]: 960.5610, found: 960.5708.

3.2.1.17. 1-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(furan-2-ylmethyl)-3-oxoisoindoline-1-carboxylic acid (**18q**)

This derivative was isolated as a yellow solid in 60% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.4; mp = 81-83 °C; $[\alpha]_D^{20}$ +76 (*c* 0.5 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2924.69 (CH str.), 1724.90 (3 x C=O), 1711 (C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.79 (d, *J* = 7.4 Hz, 1H_{aro}), 7.55–7.27 (m, 10H_{aro}), 6.34 (dd, *J*

= 6.9, 2.8 Hz, 2H_{aro}), 5.27 (t, *J* = 4.2 Hz, 1H), 5.23–4.78 (m, 6H), 4.58–4.35 (m, 2H), 3.51 (s, 1H), 2.89 (dd, *J* = 14.1, 4.5 Hz, 1H), 1.10 (s, 3H), 0.93–0.86 (m, 9H), 0.80 (s, 3H), 0.72 (s, 3H), 0.59 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.53 (C=O), 171.42 (C=O), 168.26 (C=O), 165.83 (C=O), 150.45 (C^q), 144.47 (C^q), 143.85 (2 x C^q), 142.66 (CH_{aro}), 136.51 (C^q), 132.25 (C^q), 131.88 (CH_{aro}), 128.56 (2 x CH_{aro}), 128.51 (2 x CH_{aro}), 128.08 (2 x CH_{aro}), 128.02 (CH_{aro}), 123.91 (CH_{aro}), 122.68 ($CH_{aro/trz}$), 122.37 (CH), 110.65 (CH_{aro}), 108.94 (CH_{aro}), 83.87 (CH), 66.02 (CH_2), 55.28 (CH), 47.59 (CH), 46.82 (C^q), 45.94 (CH_2), 41.77 (C^q), 41.46 (CH), 39.36 (C^q), 38.07 (CH_2), 37.86 (C^q), 37.83 (C^q), 37.06 (2 x CH_2), 36.94 (C^q), 33.94 (CH_2), 33.20 (CH_3), 32.66 (CH_2), 32.44 (CH_2), 30.80 (C^q), 29.80 (CH_2), 28.18 (CH_3), 27.69 (CH_2), 25.94 (CH_3), 23.74 (CH_3), 23.48 (2 x CH_2), 23.12 (CH_2), 18.24 (CH_2), 16.94 (CH_3), 16.65 (CH_3), 15.42 (CH_3).

3.2.1.18. ethyl2-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1-(2-ethoxy-2-oxoethyl)-3-oxoisoindoline-1-carboxylate (**18r**)

This derivative was isolated as a white solid in 98% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 97-99 °C; $[\alpha]_D^{20}$ +55 (*c* 1 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2936.99 (CH str.), 1710.10 (5 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.88–7.77 (m, 2H_{aro}), 7.61–7.45 (m, 3H_{aro}), 7.39–7.28 (m, 5H_{aro}), 5.27 (t, *J* = 3.7 Hz, 1H), 5.15–4.85 (m, 6H), 4.53 (dd, *J* = 9.5, 6.5 Hz, 1H), 4.26–4.05 (m, 2H), 4.03–3.92 (m, 2H), 3.52 (d, *J* = 17.1 Hz, 1H), 3.13 (dd, *J* = 17.0, 2.6 Hz, 1H), 2.89 (dd, *J* = 14.2, 4.4 Hz, 1H), 1.19 (td, *J* = 7.1, 2.5 Hz, 3H), 1.11–1.03 (m, 6H), 0.92–0.83 (m, 9H),0.80 (s, 3H), 0.69 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.55 (C=O), 169.08 (C=O), 169.40 (C=O), 168.92 (C=O), 165.91 (C=O), 143.85 (C^q), 143.57 (C^q), 136.54 (2 x C^q), 132.40 (2 x CH_{aro}), 131.20 (C^q), 129.52 (CH_{aro}), 128.53 (2 x CH_{aro}), 128.09 (2 x CH_{aro}), 128.03 (CH_{aro}), 123.74 (CH_{aro}), 122.40 ($CH_{aro/trz}$ + CH), 83.83 (CH), 69.23 (C^q), 66.03 (CH_2), 62.77 (CH_2), 61.12 (CH_2), 55.28 (CH), 51.17 (CH_2), 47.59 (CH), 46.84 (C^q), 45.95 (CH_2), 41.78 (C^q), 41.47 (CH), 40.38 (CH_2), 39.37 (C^q), 38.07 (CH_2), 37.83 (C^q), 36.94 (CH_2 + C^q), 33.96 (CH_2), 33.21 (CH_3), 32.67 (CH_2), 32.46 (CH_2), 30.81 (C^q), 28.18 (CH_3), 27.70 (CH_2), 25.95 (CH_3), 23.76 (CH_3), 23.48 (2 x CH_2), 23.14 (CH_2), 18.23 (CH_2), 16.96 (CH_3), 16.63 (CH_3), 15.40 (CH_3), 14.00 (2 x CH_3); HRMS (+ESI) calculated for $C_{57}H_{75}N_4O_9$ [M+H$^+$]: 959.5489, found: 959.5543.

3.2.1.19. ethyl 1-allyl-2-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-oxoisoindoline-1-carboxylate (**18s**)

This derivative was isolated as a white solid in 90% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 100-102 °C; $[\alpha]_D^{20}$ +53 (*c* 1.5 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2943.31 (CH str.), 1731.34 (2 x C=O), 1701.17 (2 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.93 (s, 1$H_{aro/trz}$), 7.80 (d, *J* = 7.4 Hz, 1H_{aro}), 7.62–7.44 (m, 3H_{aro}), 7.39–7.28 (m, 5H_{aro}), 5.26 (t, *J* = 3.7 Hz, 1H), 5.17–4.67 (m, 9H), 4.55 (t, *J* = 8.0 Hz, 1H), 4.18–4.01 (m, 2H),3.26–3.11 (m, 2H), 2.89 (dd, *J* = 13.6, 4.4 Hz, 1H), 1.14 (dt, *J* = 7.1, 3.5 Hz, 3H), 1.10 (s, 3H), 0.93–0.85 (m, 9H),0.82 (s, 3H), 0.73 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,$CDCl_3$)** δ^C 177.58 (C=O), 169.97 (C=O), 169.28 (C=O), 165.87 (C=O), 144.46 (C^q), 143.85 (C^q), 143.60 (C^q), 136.55 (C^q), 132.35 (CH=), 131.49 (C^q), 129.82 (CH_{aro}), 129.27 (CH_{aro}), 128.54 (2 x CH_{aro}), 128.10 (2 x CH_{aro}), 128.04 (CH_{aro}), 125.88 (CH_{aro}), 123.69 (CH_{aro}), 122.42 ($CH_{aro/trz}$ + CH), 120.29 (CH_2=), 83.89 (CH), 72.31 (C^q), 66.05 (CH_2), 62.57 (CH_2), 55.30 (CH), 51.23 (CH_2), 47.60 (CH), 46.85 (C^q), 45.95 (CH_2), 41.79 (C^q), 41.48 (CH), 39.38 (C^q), 38.09 (CH_2), 37.86 (C^q), 37.77 (CH_2), 37.02 (CH_2), 36.96 (C^q), 33.97 (CH_2), 33.22 (CH_3), 32.69 (CH_2), 32.48 (CH_2), 30.82 (C^q), 28.22 (CH_3), 27.71 (CH_2), 25.96 (CH_3), 23.77 (CH_3), 23.51 (2 x CH_2), 23.15 (CH_2), 18.25 (CH_2), 16.97 (CH_3), 16.69 (CH_3), 15.43 (CH_3), 14.03 (CH_3); HRMS (+ESI) calculated for $C_{56}H_{73}N_4O_7$ [M+H$^+$]: 913.5435, found: 913.5466.

3.2.1.20. ethyl2-((1-(2-(((3S,4aR,6aR,6bS,8aS,12aS,14aR,14bR)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1-(3-methoxybenzyl)-3-oxoisoindoline-1-carboxylate (**18t**)

This derivative was isolated as a white solid in 79% yield; Rf (cyclohexane/EtOAc: 60/40) = 0.5; mp = 100-102 °C; $[\alpha]_D^{20}$ +70 (*c* 1.5 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2936.46 (CH str.), 1702.26 (4 x C=O); **^{1}H NMR (300 MHz, $CDCl_3$)** δ^H 7.75, (s, 1$H_{aro/trz}$), 7.69 (d, *J* = 7.5 Hz, 1H_{aro}),7.63–7.53 (m, 2H_{aro}), 7.50–

7.42 (m, 1H$_{aro}$), 7.40–7.28 (m, 5H$_{aro}$), 6.86 (td, *J* = 7.9, 2.5 Hz, 1H$_{aro}$), 6.56 (d, *J* = 7.8 Hz, 1H$_{aro}$), 6.16 (t, *J* = 6.3 Hz, 1H$_{aro}$), 5.99 (br. s, 1H$_{aro}$), 5.27 (t, *J* = 3.9 Hz, 1H), 5.10–4.85 (m, 4H), 4.86 (s, 2H), 4.54 (t, *J* = 7.9 Hz, 1H), 4.12–4.01 (m, 2H), 3.83 (d, *J* = 14.7 Hz), 3.57 (d, *J* = 14.7 Hz, 1H), 3.49 (s, 3H), 2.89 (dd, *J* = 13.8, 4.4 Hz, 1H), 1.10 (s, 3H), 1.08–1.02 (m, 3H), 0.93–0.79 (m, 12H), 0.72 (s, 3H), 0.58 (s, 3H); **^{13}C NMR (75 MHz,CDCl$_3$)** δ^C 177.52 (C=O), 169.96 (C=O), 169.28 (C=O), 165.83 (C=O), 158.96 (C^q), 143.95 (C^q), 143.81 (C^q), 136.50 (C^q), 135.19 (C^q), 132.01 (CH$_{aro}$), 131.55 (2 x C^q), 129.28 (CH$_{aro}$), 128.86 (CH$_{aro}$), 128.50 (2 x CH$_{aro}$), 128.06 (2 x CH$_{aro}$), 127.99 (CH$_{aro}$), 125.74 (CH$_{aro}$), 123.71 (CH$_{aro}$), 122.89 (CH$_{aro}$), 122.38 (CH), 122.17 (CH$_{aro/trz}$), 114.87 (CH$_{aro}$), 113.00 (CH$_{aro}$), 83.81 (CH), 72.80 (C^q), 66.00 (CH$_2$), 62.64 (CH$_2$), 55.25 (CH), 54.97 (CH$_3$), 51.14 (CH$_2$), 47.55 (CH), 46.80 (C^q), 45.91 (CH$_2$), 41.74 (C^q), 41.43 (CH), 39.54 (CH$_2$), 39.33 (C^q), 38.04 (CH$_2$), 37.81 (C^q), 37.65 (CH$_2$), 36.90 (C^q), 33.92 (CH$_2$), 33.18 (CH$_3$), 32.64 (CH$_2$), 32.43 (CH$_2$), 30.78 (C^q), 28.17 (CH$_3$), 27.66 (CH$_2$), 25.92 (CH$_3$), 23.73 (CH$_3$), 23.45 (2 x CH$_2$), 23.10 (CH$_2$), 18.20 (CH$_2$), 16.92 (CH$_3$), 16.63 (CH$_3$), 15.38 (CH$_3$), 13.84 (CH$_3$); HRMS (+ESI) calculated for $C_{61}H_{77}N_4O_8$ [M+H$^+$]: 993.5697, found: 993.5744.

3.2.1.21. ethyl1,2-bis((1-(2-(((3S,4aR,6aR,6bS,8aS,12aR,14aS,14bS)-8a-((benzyloxy)carbonyl)-4,4,6a,6b,11,11,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-3-oxo-2,3-dihydro-1H-isoindole-1-carboxylate (**18v**)

This derivative was isolated as a white solid in 80% yield; Rf (cyclohexane/EtOAc: 50/50) = 0.4; mp = 143-145 °C; $[\alpha]_D^{20}$+63 (*c* 0.9 mg/mL, CH_2Cl_2); **IR (ν_{max}/cm^{-1})** 2938.94 (CH str.), 1728.35 (6 x C=O);**^{1}H NMR (300 MHz, CDCl$_3$)** δ^H 7.92 (d, *J* = 6.6 Hz, 1H$_{aro}$), 7.73–7.61 (m, 2H$_{aro}$), 7.46–7.28 (m, 11H$_{aro}$), 6.31 (d, *J* = 16.1 Hz, 1H$_{aro}$), 5.26 (t, *J* = 3.6 Hz, 2H), 5.24–4.99 (m, 7H), 4.85 (t, *J* = 3.8 Hz, 2H), 4.67 (dd, *J* = 15.8, 4.5 Hz, 1H), 4.60–4.33 (m, 2H), 4.30–4.05 (m, 3H), 3.89 (dd, *J* = 15.3, 4.3 Hz, 1H), 2.92 (dd, *J* = 13.6, 4.4 Hz, 2H), 1.11 (s, 6H), 0.94–0.80 (m, 24H), 0.77–0.67 (m, 6H), 0.61 (s, 6H); **^{13}C NMR (75 MHz,CDCl$_3$)** δ^C 177.55 (2 x C=O), 169.68 (C=O), 169.61 (C=O), 166.61 (C=O), 166.04 (C=O), 144.52 (C^q), 143.85 (2 x C^q), 142.98 (C^q), 140.25 (C^q), 136.53 (2 x C^q), 132.45 (CH$_{aro}$), 130.98 (C^q), 129.39 (CH$_{aro}$), 128.53 (4 x CH$_{aro}$), 128.09 (4 x CH$_{aro}$), 128.03 (2 x CH$_{aro}$), 126.01 (CH$_{aro}$), 123.51 (2 x CH$_{aro/trz}$), 123.26 (CH$_{aro}$), 122.38 (2 x CH), 84.31 (CH), 83.33 (CH), 72.40 (C^q), 66.04 (2 x CH$_2$), 62.92 (CH$_2$), 55.24 (2 x CH), 51.03 (2 x CH$_2$), 47.59 (2 x CH), 46.84 (2 x C^q), 45.97 (2 x CH$_2$), 41.80 (2 x C^q), 41.48 (2 x CH), 39.37 (2 x C^q), 38.08 (2 x CH$_2$), 37.87 (C^q), 37.74 (C^q), 37.23 (CH$_2$), 36.95 (2 x C^q), 33.96 (2 x CH$_2$), 33.23 (2 x CH$_3$), 32.68 (2 x CH$_2$), 32.46 (2 x CH$_2$), 30.81 (2 x C^q), 30.11 (CH$_2$), 28.20 (2 x CH$_3$), 27.72 (2 x CH$_2$), 25.96 (2 x CH$_3$), 23.76 (2 x CH$_3$), 23.50 (4 x CH$_2$), 23.14 (2 x CH$_2$), 18.27 (2 x CH$_2$), 16.98 (2 x CH$_3$), 16.63 (2 x CH$_3$), 15.46 (2 x CH$_3$),14.10 (CH$_3$); HRMS (+ESI) calculated for $C_{95}H_{125}N_7O_{11}Na$ [M+Na$^+$]: 1562.9329, found: 1562.9335.

3.3. Antibacterial activity

In the present study, the antimicrobial activity of the starting product **OA-1** and its derivatives **18a-v** was screened by agar disc diffusion method according to the protocol described by Dbeibia et al. (2022) [79] against four bacteria, namely *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Salmonella thyphimurium* ATCC 14080 and *Pseudomonas aeruginosa* ATCC 27853. The inoculums of the microorganisms were adjusted to 0.1 at OD600 and then streaked onto Muller Hinton (MH) agar plates using a sterile cotton mop. Sterile filter discs (diameter 6 mm, Biolife Italy) were placed at the surface of the appropriate agar mediums and 20 µL of the product was dropped onto each disc. Tetracycline (10 mg/mL; 10 µL/disc) was used as reference antibiotics. After incubation at 37 °C for 24h, the antibacterial activities were evaluated by measuring an inhibition zone formed around the disc. Each assay was performed in triplicate. The minimum inhibitory concentration (MIC) was evaluated as recommended by Dbeibia et al. (2022) [79]. Briefly, serial dilutions of the synthesized compounds (3.9–2000 µg/mL) were filled in 96 U bottomed-wells plates (Nunc, Roskilde, Denmark) with MH broth and the target bacteria. The treated plates were left to incubateovernight at 37 °C. The MIC was reported as the lowest concentration of the sample that did not allow the growth of microorganisms and do not show visible turbidity of the broth medium. The minimum bactericidal concentration (MBC) was evaluated by transferring 10 µL from the well showing no bacteria growth after MIC assay, on MH agar. After 24h period of incubation at 37 °C,

the bacterial growth was examined and the MBC was determined as the lowest concentration of the sample having bactericidal activity.

3.4. Molecular docking procedure

Molecular docking studies were performed by using Auto Dock 4.2 program package [80]. The optimization of all the geometries of ligands was carried out by ACD (3D viewer) software (http://www.filefacts.com/acd3d-viewer-freeware-info) and the three dimensional structure of PDB (PDB: 5F7V) [81] was obtained from the RSCB protein data bank (https://www.rcsb.org/). Before docking, the water molecules have been erased and the missing hydrogens besides to Gasteiger charges were then added to the system during the receptor preparation input file. Then, the AutoDock Tools were used for the preparation of all ligands and protein files (PDBQT). Pre-calculation of grid maps was performed by Auto Grid for saving a lot of time during docking procedure and the docking calculation was carried out by a grid per map with 40 × 40 × 40 A° points of all PDB used besides to the grid-point spacing of 0.375 A °, that was centered on the receptor structure in order to determine the active site and the visualization and analysis of interactions were performed using Discovery Studio 2017R2 (https://www.3dsbiovia.om/products/collaborative-science/biovia-discovery-studio/). Further, all molecular docked models for the cavities 3D were prepared via PyMOL viewer v. 0.99 [82].

3.5. Statistical analysis

Statistical analysis was carried out using Graph Pad Prism 7.0 (Graph Pad Software Inc., CA, USA). The experimental data of the antibacterial activity expressed in inhibition zone are presented as mean ± standard error of the mean (SEM). Student's t-test was used to assess the difference between two groups. For significant differences among three or more groups, one-way ANOVA with post hoc analysis was performed.

4. Conclusions

In summary, oleanolic acid (OA-1) isolated from olive pomace (*Olea europaea* L.) was used as a starting material to prepare a series of new (OA-1)-phtalimidines coupled 1,2,3-triazole derivatives by application of the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction. All the synthesized compounds were obtained in good yields (70–98%). The designed click products were assessed for their antibacterial activity toward four bacterial strains (gram-positive: *S. aureus* and *L. monocytogenes*; gram-negative: *S. thyphimurium* and *P. Aeruginosa*). Significant antibacterial activities were observed notably against *L. Monocytogenes*. Interestingly, compound 18g exhibited the highest activity toward this bacterium (MIC = 9.48 µmol/L) followed by derivatives 18d (MIC = 9.56 µmol/L) and 18h (MIC = 9.89 µmol/L), compared to the reference antibiotic Tetracycline. In silico molecular docking study revealed that the chosen lead compounds 18c, 18d, 18h and 18k can fit in well with the binding cavity of ABC substrate-binding protein Lmo0181 from *L. monocytogenes*. This study lays a strong starting pointfor future efforts of optimization and expansion of the antibacterial spectrum of the (OA-1)-Phtalimidines derivatives against gram-positive bacteria, as well as gaining deeper insights into the mechanism of action and resistance potential of thesenewly developed semi-synthetic compounds.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

Author Contributions: Conceptualization, Methodology and Data curation, G.L.; Software, Visualization, Validation, M.H.; Writing—Original draft preparation, G.L. and M.H.; Conducting the testing of antibacterial activity, A.D. and A.M.; Validation, A.M. and A.H.H.; Supervision, A.R., A.M.L. and A.Da.; Writing- Reviewing and Editing, H.B.J. and M.O. The manuscript was a collaborative effort by all authors. The final version of the manuscript has received approval from all the authors.

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Sample Availability: Samples of the compounds are available from the authors upon request.

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