Synthesis, Antibacterial, and Cytotoxicity Evaluation of Oleanolic Acid-4-aminoquinoline Based Hybrid Compounds

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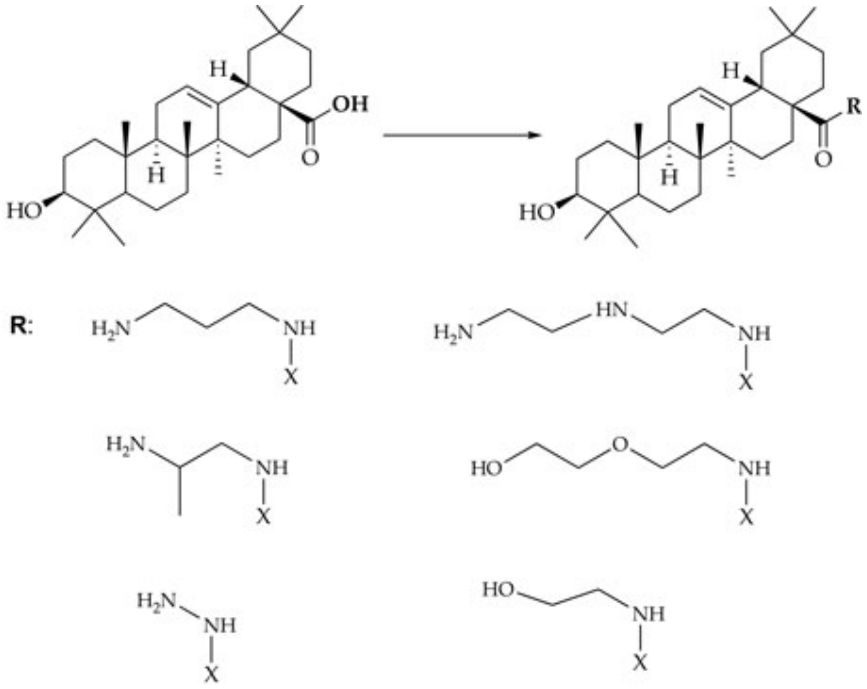
Graphical abstract



Synthesis of oleanolic acid-4-aminoquinoline hybrids.

Abstract

Aim: The study aims to prepare a class of oleanolic-based compounds.

Background: Conventional drugs used to treat infectious diseases suffer from limitations such as drug toxicity and drug resistance. The resistance of microbes to antimicrobial agents is a significant challenge in treating microbial infections. Combining two or more drugs with different modes of action to treat microbial infections results in a delay in developing drug resistance by the microbes. However, it is challenging to select the appropriate drugs for combination therapy due to the differences in stability and pharmacokinetic profile of the drugs. Therefore, developing hybrid compounds using the existing drugs is a promising approach to design effective antimicrobial agents.

Objectives: To prepare oleanolic-based hybrid compounds followed by characterization, in vitro antibacterial and cytotoxicity evaluation.

Methods: Oleanolic acid-4-aminoquinoline-based hybrid compounds were synthesized via esterification and amidation. The compounds were characterized using FTIR, NMR, and UHPLC-HRMS. Oleanolic acid (OA) was isolated from the flower buds of Syzygium aromaticum (L.) Merr. & L.M.Perry, a species from Kingdom Plantae, order Mytales in the Myrtaceae family. Antibacterial activity was determined against selected strains of bacteria using the microdilution assay and cytotoxicity activity was assessed using the sulforhodamine B assay against selected cancer cell lines.

Results: The synthesized hybrid compounds exhibited antibacterial activity against the Gram-positive bacteria Enterococcus faecalis (ATCC13047), Bacillus subtilis (ATCC19659), Staphylococcus aureus as well as Gram-negative bacteria, Klebsiella oxytoca (ATCC8724), Escherischia coli (ATCC25922), and Proteus vulgaris (ATCC6380) with minimum inhibitory concentrations of 1.25 mg/mL compared to oleanolic acid (2.5 mg/mL). Compounds 13 and 14 displayed cytotoxicity in vitro against the cancer cell lines (MCF-7 and DU 145) compared to the oleanolic acid (IC50 > 200 μM).

Conclusion: Modification of C28 of OA enhanced its biological activity.

Keywords: *Syzygium aromaticum*; oleanolic acid; 4-aminoquinoline derivatives; antibacterial activity; cytotoxicity; , hybrid compounds.

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1. Introduction

Pathogenic microorganisms affect quality of life. Every year, a large number of people around the globe succumb t0 bacterial infections [1,2]. Resistance to antimicrobial agents has proven to be a major challenge in treating infections [3,4]. According to the Centre for Disease Control and Prevention (CDC), approximately 2 million people in the USA are reported to contract antibiotic-resistant infections yearly, with over 23,000 dying as a result thereof [5]. According to the World Health Organisation (WHO), therapy using a combination of two or more drugs with different modes of action can delay or prevent the development of drug resistance [6]. The development of an effective combination therapy is expensive and drug-drug interactions may cause additive toxic side effects [7]. Another challenging problem is the choice of drugs and dosages for combination therapy due to the

differences in stability, solubility, and pharmacokinetic profile of the drugs. Therefore, modification of existing drugs through the synthesis of hybrid compounds would appear a promising approach. Combining two or more active pharmacophores into a single molecule (hybrid compound) can overcome combination therapy while enhancing overall potency [8, 9].

Plant-based natural products have been reported to exhibit biological activity. Oleanolic acid (**1**) (3b-hydroxyolean-12-en-28-oic acid, OA) is a plant-derived natural product and a pentacyclic triterpenoid, which has been reported to inhibit the growth of various bacterial pathogens by targeting the cell envelope [10–12]. OA is found in among other plants, S*yzygium aromaticum* (L.) Merr. and Perry[13, 14]. The plant possesses a variety of biological activities such as anticancer [15,16], antioxidant [17,18], antifungal [19,20], and antidiabetic activity [21]. However, OA's large molecular weight and low solubility in aqueous solutions limits its bioavailability and therapeutic application in clinical medicine [18,22]. Therefore, in order to improve the activity and bioavailability of OA, modification of its structure is required.

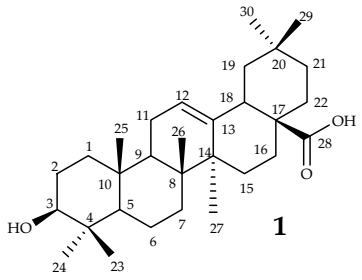

Figure 1: Structure of oleanolic acid.

The modification of triterpenoids at C-28 COOH position through amination and esterification has been found to enhance this compound's biological activity [12,23–25]. Also, the introduction of nitrogen-containing heterocyclic rings to the pentacyclic triterpenoids, has been reported to improve biological activity [26–28]. Nitrogen-containing heterocyclic compounds such as 4-aminoquinoline derivatives have been reported to exhibit a wide range of pharmacological activities such as anti-malarial, anti-bacterial, anti-fungal, and rheumatoid arthritis [8,29,30]. Furthermore, hybridizing 4-aminoquinoline derivatives with selected compounds have resulted in potent antibacterial [31–34] and anticancer drugs [34–38].

Due to the potency of hybridizing 4-aminoquinoline derivatives with selected compounds, the aim of the study was to prepare hybrid compounds containing selected 4-aminoquinoline derivatives and OA via C28. The compounds were characterized by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), carbon nuclear magnetic resonance spectroscopy (^{13}C-NMR), Ultra high pressure liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS), and Fourier Transform Infrared

Spectroscopy (FTIR). Biological activity included the determination of antibacterial activity as well as *in vitro* cytotoxicity.

2. Materials and methods

2.1. General experimental procedures

All chemicals and reagents used were purchased from Sigma-Aldrich (Johannesburg, South Africa) and used without further purification unless and otherwise specified. The solvents used for synthesis were of the highest grade were dried over molecular sieves with the pore size of 4 Å and particle size of 4-8 Mesh. The fingerprint identification of specific functional groups was achieved by assigning of group frequencies (cm^{-1}) of particular peaks within the IR spectra. The IR spectra were obtained from Perkin Elmer model 100 Hz and the percentage transmittance was recorded against the wave number (cm^{-1}) for the reported signals in the range of 4000- 400 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Nuclear Magnetic Resonance Spectrometer 400 MHz at room temperature using deuterated chloroform ($CDCl_3$). The chemical shifts (δ) are reported in ppm relative to internal solvent peaks and the coupling constants (*J*) were measured in Hertz (Hz). The high resolution mass spectra were recorded using an Ultra High Pressure Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS) spectrometer (Kyoto, Japan).

2.2. Plant identification

The dried flower buds of *Syszygium aromaticum* were purchased from the spice market in Durban, South Africa. The plant was authenticated by Mr. Pravin Poorun, a senior plant taxonomist and a botanist from the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville. A voucher specimen (number OO4) is deposited at the University herbarium.

Oleanolic acid was isolated from cloves, and flower buds of *Syzygium aromaticum* using a standard protocol validated in-house [39,40]. The plant material was dried and ground to a powder using a mortar and pestle. The sample (500 g) was sequentially extracted with different organic solvents (2000 mL) of increasing polarity; n-hexane, ethyl acetate, dichloromethane and methanol. The mixture was placed on a rotating shaker for a period of about two weeks. The supernatant was filtered using Whatman filter paper. The filtrate was concentrated on a rotary evaporator at reduced pressure. Thereafter, the concentrated extracts were collected in a pre-weighed beaker and allowed to air dry for complete evaporation of the extracting solvents then air dried under a fume hood. The ethyl acetate extract containing mixtures of eugenol (EU), OA and maslinic acid (MA) was purified by column chromatography using silica gel (Merck, silica gel 60 F_{254}: 0.063-0.200 mm) with hexane: ethyl acetate solvent systems, 7:3 for OA and 6:4 for MA. This yielded EU, OA and MA, respectively which were

further purified by recrystallization from methanol. The obtained compounds were confirmed by spectroscopic analysis using Mass spectrometry (MS), Melting Point (m.p), Fourier-Transform Infrared Spectroscopy (FT-IR), and ^{1}H-/^{13}C-Nuclear Magnetic Resonance (NMR) spectroscopy. The isolated OA ($C_{30}H_{48}O_3$), was obtained as a white amorphous powder 7.68 g (17.3%) with R_f 0.45, Mz+ 456, m.p. 240∘C. IR (ν_{max} cm^{-1}): 3443 (-OH), 2941 - 2870 (aliphatic -CH), 1693 (-C=O), 1468 (-C=C), 1031-998 (-C-O) respectively. ^{1}H-NMR (400 MHz, $CDCl_3$) indicated signals of an olefinic proton H-12 at 5.35 cm^{-1}, carbonyl proton H-3 at 2.19 and 7 methyl groups (CH_3) at 0.87, 0.78, 0.91, 0.81, 1.21, 0.89 and 0.89 ppm which confirmed the oleanane skeleton [41]. ^{13}C-NMR (C1–C30, ppm): 33.64, 27.48, 79.03, 41.25, 55.24, 22.97, 35.3, 39.9, 49.8, 37.03, 25.88, 123.07, 143.22, 41.25, 29.71, 27.48, 55.24, 41.78, 47.64, 30.67, 39.43, 31.45, 18.43, 18.32, 23.46, 17.18, 23.63, 172.93, 27.19, and 27.48.

2. Synthesis of 4-aminoquinoline derivatives

2.1. Synthesis of N-(3-aminopropyl)-7-chloroquinolin-4-amine (3)

Compound **3** was prepared by using the modified method of Sunduru et al. (Scheme 1) [42]. A solution of 4,7-dichloroquinoline (1 g, 5.05 mmol) in 1,3-diaminopropane (1.9 mL, 22.7 mmol) was refluxed for 24 h at 90 ℃ while stirring, and the reaction was monitored by TLC using methanol:Triethanolamine (TEA): hexane (6:3:1) as eluent solvent system to ensure the completion of reaction. The reaction was thereafter allowed to cool to room temperature. The mixture was diluted with 60 mL dichloromethane (DCM) (20 mL x 3) and the resulting mixture was washed with sodium hydroxide (NaOH) (1 M, 10 mL x 3) followed by 10 mL brine, which resulted in an aqueous layer, an organic layer, and a white course particulate precipitate. The organic layer was filtered through a cotton plug and concentrated using a rotary evaporator. Compound **3** (Yield 62 %, m.p 129-131℃, R_f value 0.34) was obtained as pale yellow crystals. IR ν_{max}: 3357-3279 cm^{-1} (N-H), 2947 cm^{-1} (CH), 601 cm^{-1} (C-Cl) and 1582 cm^{-1} (C=C).

2.2. Synthesis of N-(2-aminopropyl)-7-chloroquinolin-4-amine (4)

Using a similar procedure as described for compound **3** (Scheme 1), 4,7-dichloroquinoline (1 g, 0.05 mmol) and 1,2-diaminopropane (1.9 mL, 22.7 mmol) was heated to reflux at 90 ℃ for 24 h while stirring, and the reaction was monitored by TLC using MeOH:TEA:EtOAc (6:3:1) as eluent solvent system to ensure the completion of reaction. The reaction was allowed to cool to room temperature. The work-up method was similar to compound **3.** Compound **4** (Yield 66 %, m.p 128–130 ℃, R_f value 0.64) was obtained as pale yellow crystals. IR ν_{max}: 3366 (N−H), 2901 (C−H), 1587(C=C) and 756.5 (C−Cl).

2.3. Synthesis of N-(2-(2-Aminoethylamino) ethyl)-7-chloroquinoline-4-amine (5)

Compound **5** was prepared using the modified procedure described by Musonda et al. [43]. A solution of 4,7-dichloroquinoline (1.00 g, 5.05 mmol) in diethelenetriamine (1.05 g, 10.1 mmol) was heated to reflux at 85 ℃ for 24 h while stirring. The reaction was monitored by TLC using MeOH: TEA: Hex (6:3:2) as eluent solvent system to ensure the completion of reaction, then allowed to cool to room temperature. The mixture was basified with 20 mL 1 M NaOH, extracted with 60 mL hot EtOAc (20 mL x 3) and the resulting mixture was washed with Na_2SO_4 (10 mL). The separated organic layer was filtered and concentrated in a rotary evaporator and then later purified with column chromatography. IR ν_{max}: 3302 cm^{-1} (NH), 2802 cm^{-1} (C−H), 1579 cm^{-1} (C=C), and 647 cm^{-1} (C−Cl) stretch at 756.5 cm^{-1} [43], [44]. Yield 85 %, m.p 112–114 ℃, R_f value 0.56.

2.4. Synthesis of 1-(7-Choloroquinolin-4-yl) hydrazine (6)

A solution of 4.7- dichloroquinoline (1 g, 5. 05 mmol) and hydrazine (0.75 mL, 30.30 mmol) in 3 mL ethanol as a solvent was refluxed at 95 ℃ for 24 h. The reaction was monitored by TLC using toluene: EtOAc: MeOH (6:3:1) as eluent solvent system to ensure the completion of the reaction. The obtained solution was filtered and allowed to dry in fume-cardboard after which it was recrystallized with 10 mL ethanol. Compound **6** (Yield 72 %, m.p 129–132 ℃, R_f value 0.64) was obtained as pale yellow crystals. IR ν_{max}: 3278 (NH), stretch for alkene at 3120 (C-H), 2945 (C-H), 1550 (C=C), and 643 (C-Cl) [45].

2.5. Synthesis of 2-(7-chloroquinolin-4-ylamino) ethanol (7)

4.7-Dichloroquinoline (1000 mg, 5.05 mmol) was dissolved in ethanolamine (3.05 mL). The reaction was refluxed over a period of 24 h at 8 ℃. The reaction was monitored by TLC using toluene: EtOAc: MeOH (6:3:1) as eluent solvent system to ensure the completion of reaction. After 24 h, the reaction was allowed to cool to room temperature and 30 mL of distilled water was added into the solution and the resultant was filtered. The solids were dried at room temperature after which it was recrystallized with 20 mL of methanol. IR ν_{max}: 3300 cm^{-1} (NH), 2802 cm^{-1} (C−H), 1580 cm^{-1} (C=C), and 750 cm^{-1} (C−Cl) stretch at 756.5 cm^{-1}[46]. Yield: 75%, m.p: 221–225 °C (lit 220–222 ℃) [29], [47]

2.6. Synthesis of 2-(2-(-chloroquinolin-4-ylamino)ethoxy)ethanol (8)

4.7-Dichloroquinoline (1000 mg, 5.05mmol) was dissolved in 2(2-Aminoethoxy) ethanol (3.05 mL). The reaction was refluxed over a period of 24 h at 85 ℃. Thereafter, the reaction was allowed to cool to room temperature and 30 mL of distilled water was added and the resultant was filtered, with the solids being dried at room temperature. An aliquot of 1 mL of methanol and 5 mL of ethyl acetate was

used to boil the solids. Ice was used to cool the solids after collection. Compound **8** (Yield 75%, m.p 122–124 ℃, R_f value 0.62)) [46] was obtained as brownish orange crystals. IR v_{max}: 3310 (N-H), 2803 (C-H), 1580 (C=C), 1108 (C-N), 1050 (C-O) and 530 (C-Cl).

2.7. Synthesis of oleanolic acid-4-aminoquinoline based hybrid compounds (9)-(12).

Oleanolic acid (200 mg, 0.4 mmol) and N-(3-aminopropyl)-7-chloroquinolin-4-amine (103 mg, 0.4 mmol) was dissolved in 5 mL of DMSO. N-Hydroxysuccinimide (HSU) (50.40 mg, 0.4 mmol) and N,N'-Dicyclohexylcarbodiimide (DCC) (99.39 mg, 0.5 mmol) portions were added and stirred at room temperature for 10 min after which it was heated to reflux at 120 ℃ for two days. The reaction was monitored by TLC to ensure the completion of reaction, after which the resultant solution was diluted with DCM (20 mL) and the mixture was washed with 30 mL of ice cold water to afford an organic and aqueous layer. The organic layer was separated and dried over anhydrous sodium sulphate, filtered and concentrated on the rotary evaporator to obtain a light brown powder. The crude compound was purified by column chromatography using silica gel (Merck, silica gel 60 F_{254}: 0.063-0.200 mm). The column was eluted with EtOAc: MeOH (9:1) to obtain compounds **9-12** (Scheme 1).

*2.7.1. Hybrid compound **(9)***

Yield 54%, m.p 131–133 ℃, R_f value 0.42). IR v_{max} 3296 (NH), 3076 (OH), 2932-2873 (aliphatic CH), 1626(C=O), 1545(C=C), 1337(C-N). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.28-8.26 (d, *J*=8.0 Hz, 1H, H-40), 7.32 (s, 1H, C-48), 7.18-7.16 (d, *j*=8.0 Hz, 1H, H-38), 7.08-7.06 (d, *j*=8.0 Hz, 1H, H-33), 5.66-5.64 (d, *j*=8.0 Hz, 1H, H-41), 5.28 (t, *J*=4.0 Hz, 1H, H-13) 4.06-4.00 (q, *j*=8.0 Hz, 3H, H-2 & 47), 3.39(s, 1H, H-48), 3.17-3.13 (dd, *j*=4.0 Hz, 2H, H-45), 2.48-2.43 (dd, *j*=4.0 Hz, 1H, H-18), 2.19 (s, 1H, H-32). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 177.13 (C27), 155.23 (C42), 152.43 (C40), 148.21 (C36), 144.73 (C12), 135.43 (C34), 129.11 (C35), 125.77 (C33), 122.53 (C38), 121.34 (C13), 119.41 (C37), 114.24 (C41), 78.97 (C2). MS (ESI^+) 673.44 calculated for $C_{42}H_{60}ClN_3O_2$ $[M+H]^+$, found 673.33 (Figure 2).

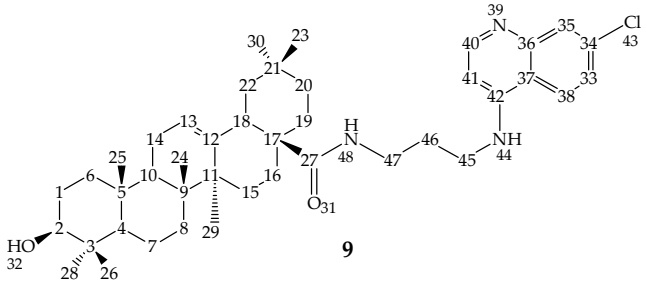

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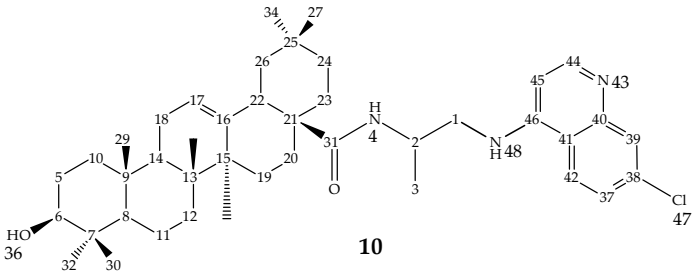
Figure 2: Compound 9

*2.7.2. Hybrid compound **(10)***

Using a similar procedure described for compound **9**, compound **10** (Figure 3) was obtained as a white powder (Yield 51%, m.p 122–124 ℃, R_f value 0.52). IR ν_{max} 3546 (NH), 3359 (OH), 2928-2857 (CH aliphatic), 1712 (C=O), 1637(C=C alkene), 1456 (C=C aromatic), 1223(C-N), 626 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.92 (s, 1H, H-4), 7.62 (s, 1H, H-39), 7.56-7.54 (d, *J*= 8.0 Hz, 1H, H-42), 7.41-7.39 (d, *J*=8.0 Hz, 1H, H-37), 5.64-5.62 (d, *J*=8.0 Hz, 1H, H-45), 5.28 (t, *J*=4.0 Hz 1H, H-17), 4.45 (s, 1H, H-48), 4.32-4.30 (d, *J*=8.0 Hz, 1H, H-2), 3.17- 3.13 (dd, *J*=4.0 Hz, 1H, H-6), 2.48- 2.44(dd, *J*=4.0 Hz, 1H, H-22), 2.19 (s, 1H, H-36). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 177.03 (C31), 155.75 (C38), 150.23 (C42), 148.23 (C40), 144.00 (C16), 133.75 (C46), 128.43 (C47), 126.41 (C45), 122.50 (C44), 121.13 (C17), 119.23 (C39), 114.34 (C43), 78.96 (C6). MS (ESI^+) 673.44 calculated for $C_{42}H_{60}ClN_3O_2$ $[M+H]^+$, found 671.09.

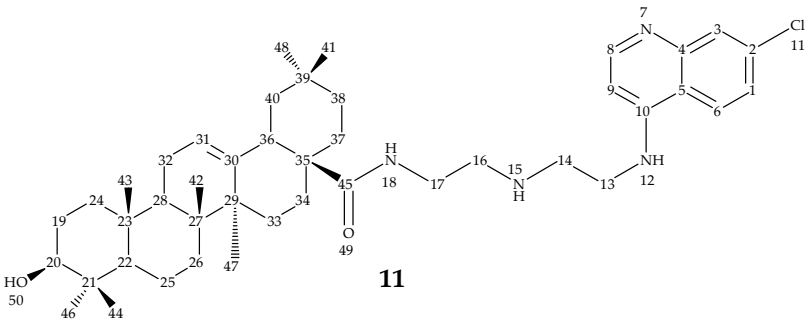


10

Figure 3: Compound **10**

*2.7.3. Hybrid compound **(11)***

By using a similar procedure described for compound **9**, compound **11** (Figure 4) was obtained as a white powder (yield 62%, m.p 128–130 ℃, R_f value 0.52). IR ν_{max} 3356 (NH), 3265 (OH), 2943-2857 (CH aliphatic), 1712 (C=O), 1637 (C=C alkene), 1456 (C=C aromatic), 1223(C-N), 626 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.74-7.72 (d, *J*=8.0 Hz, 1H, H-8), 7.14(s, 1H, H-3), 7.04-7.02(d, *J*=8.0 Hz, 1H, H-6), 6.74-6.72(d, j=8.0, 1H, H-1), 6.42-6.40(d, J=8.0, 1H, H-9), 5.28 (t, J=4.0, 1H, H-31), 4.45 (s, 1H, H-12), 4.04 (m, 4H, H-13& H-17), 3.66 (q, J=8.0, 4H, H-14& 16), 3.40 (s, 1H, H-18), 3.14 (dd, J=8.0, 1H, H-36), 2.45 (dd, 4.0 Hz, 1H, H-20), 1.98 (s, 1H, H-50). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 177.07 (C45), 154.65 (C10), 151.30 (C8), 148.44 (C4), 144.75 (C30), 134.50 (C2), 129.13 (C3), 127.41 (C1), 122.50 (C6), 121.99 (C31), 119.09 (C5), 113.21 (C9), 78.96 (C20), 48.08 (C13), 47.57 (C17), 46.81 (C14), 46.23 (C16). MS (ESI^+) 702.46 calculated for $C_{43}H_{63}ClN_3O_2$ $[M+H]^+$, found 701.38.

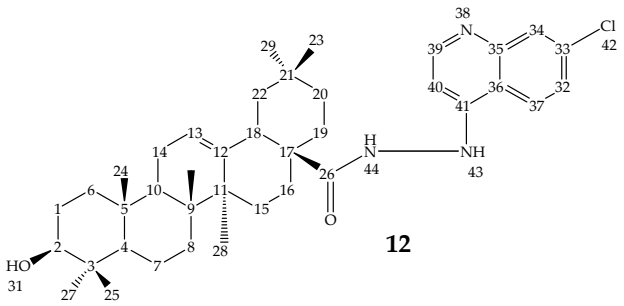


11

Figure 4: Compound **11**

*2.7.4. Hybrid compound **(12)***

A similar procedure described for compound **9** was used to obtain compound **12** (**Figure 5**). This compound was obtained as a yellowish white powder (yield 66 %, m.p 114–115 ℃, R_f value 0.33). IR ν_{max} 3548 (NH), 3383 (OH), 2929-2857 (CH aliphatic), 1709(C=O), 1631(C=C alkene), 1455(C=C aromatic), 1217(C-N), 628 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.06-8.04 (d, *J*=8.0 Hz, 1H, H-39),7.89(s,1H, C34), 7.62-760 (d, J=8.0 Hz, 1H, C-37), 7.44-7.42 (d, *J*= 8.0 Hz, 1H, H-32), 5.65-5.63 (d, *J*=8.0 Hz, 1H, H-40), 5.28 (t, *J*=4.0 Hz 1H, H-13), 3.40 (s, 1H, H-44), 3.17-3.13 (dd, *J*=4.0 Hz, 1H, H-2), 2.48-2.43 (dd, *J*=4.0 Hz, 1H, H-18), 2.10 (s, 1H, H-31). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 181.52 (C26), 152.12 (C39), 149.97 (C41), 148.24 (C35), 144.74 (C12), 135.43 (C33), 129.65 (C34), 127.61 (C32), 122.52 (C37), 121.99 (C13), 118.42 (C36), 113.65 (C40), 78.97 (C2). MS (ESI^+) 631.39 calculated for $C_{39}H_{54}ClN_3O_2$ $[M+H]^+$, found 631.35.



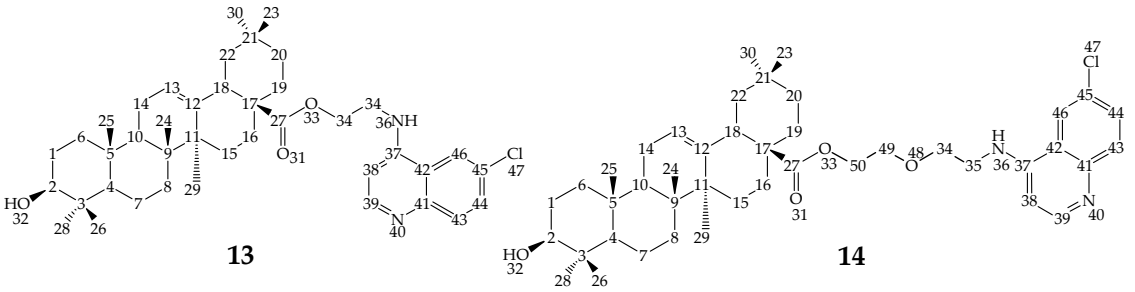
12

Figure 5: Compound **12**

2.8. General procedure for synthesis of hybrid compounds (13) and (14).

Oleanolic acid (400 mg, 0.8 mmol) and 2-(7-chloroquinolin-4-ylamino) ethanol (195.02 mg, 0.8 mmol) was dissolved in 5 mL of DMSO. 4-Dimethylaminopyridine (DMAP) (97.74 mg, 0.8 mmol) and DCC (181.56 mg, 0.88 mmol) portions were added and the mixture stirred at room temperature for 10 min, where after, it was heated to reflux at 120 ^{0}C for 28 h. The reaction was monitored using TLC to ensure the completion of reaction, after which the obtained solution was diluted with DCM (20 mL) and the

mixture was washed with 30 mL of ice cold water to afford an organic and aqueous layer. The organic layer was collected and dried over anhydrous sodium sulphate, filtered and concentrated using a rotary evaporator to obtain a light brown powder. The crude was purified by column chromatography using silica gel (Merck, silica gel 60 F_{254}: 0.063-0.200 mm). The column was eluted with EtOAc: MeOH (9:1) to obtain compounds **13** and **14.**



13 **14**

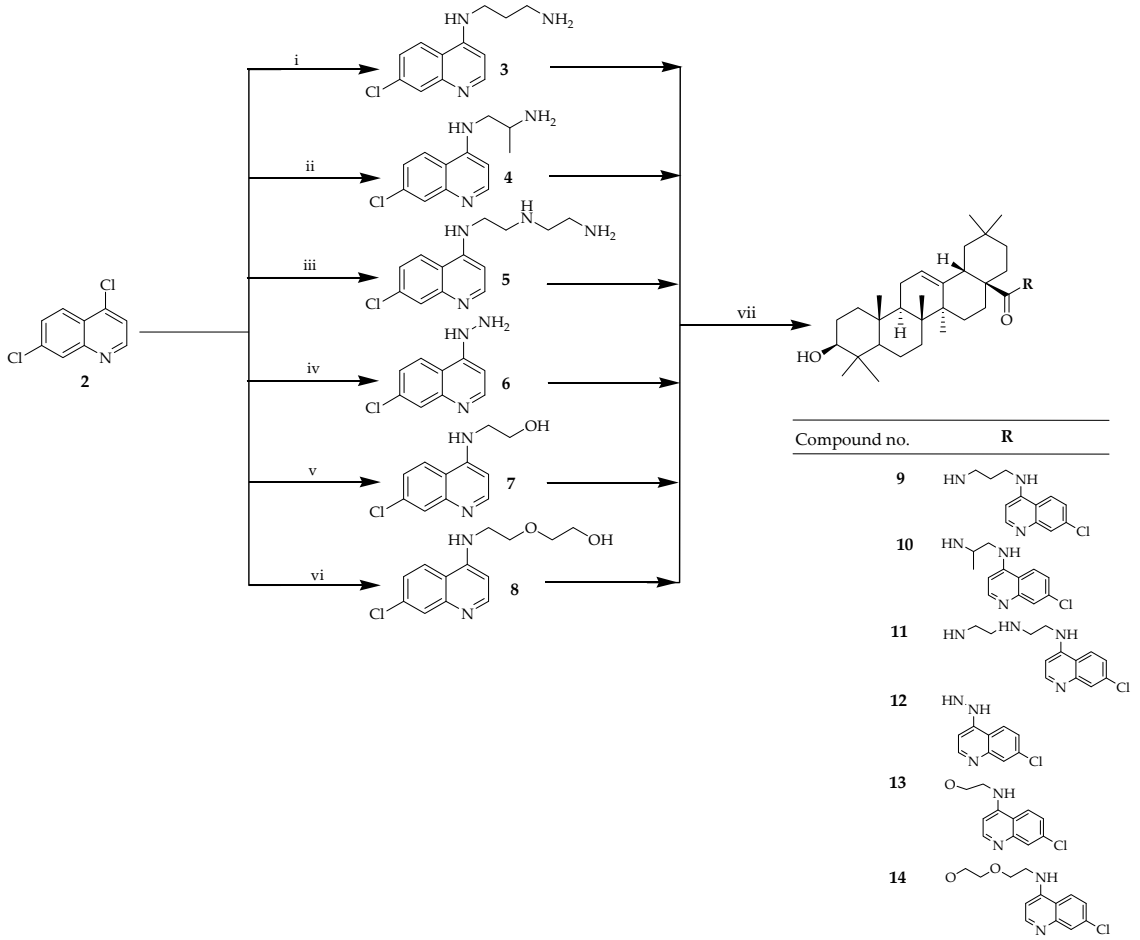
Figure 6: Compound **13 and 14**

*2.8.1. Hybrid compound **(13)***

Yield 54%, mp 131–133 ^{0}C, R_f value 0.43). IR ν_{max} 3450 (NH), 3307 (OH), 2943-2897 (CH aliphatic), 1694 (C=O), 1468(C=C alkene), 1398 (C=C aromatic), 1274(C-N), 660 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.94-7.92 (d, *J*=8.0 Hz, 1H, H-39), 7.78(s, 1H, H-46), 7.58-7.56 (d, *J*=8.0 Hz, 1H, H-44), 5.64-5.62(d, *J*=8.0 Hz, 1H, H-38), 5.38(t, *J*=4.0 Hz,1H, H-13) 4.45(s, 1H, H-36), 4.34-4.29(dd, *J*=8.0 Hz, 1H, H-34), 4.05-4.00 (dd, *J*=8.0 Hz, 2H, H-35), 3.17-3.13(dd, *J*=4.0 Hz, 1H, H-2), 2.48-2.44(dd, *J*=4.0 Hz, 1H, H-18). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 177.05 (C27), 152.43 (C37), 149.20 (C39), 145.74 (C41), 144.51 (C12), 130.50 (C45), 130.21 (C44), 130.01 (C43), 122.50 (C42), 121.72 (C13), 107.50 (C46), 103.43 (C38), 78.96 (C2), 58.44 (C34), 55.13 (35). MS (ESI^+) 660.41 calculated for $C_{41}H_{57}ClN_2O_3$ $[M+H]^+$, found 660.38.

*2.8.2. Hybrid compound **(14)***

Yield 51%, m.p 132–133 ^{o}C, R_f value 0.45). IR ν_{max} 3535 (NH), 3391 (OH), 2932-2855 (CH aliphatic), 1704 (C=O), 1626 (C=C alkene), 1451 (C=C aromatic), 1274 (C-N), 628 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.10-7.08 (d, J=8.0 Hz, 1H, H-39), 6.85-6.83 (d, J = 8.0 Hz, 1H, H-43), 6.80 (s, 1H, H-46), 6.51-6.50 (d, J = 8.0 Hz, 1H, 44-H), 5.72-5.70 (d, J=8.0, 1H, H-38), 5.37 (t, J = 4.0 Hz, 1H, H-13), 4.16 (q, 4H, H-50), 3.19 (s, 1H, H-36), 3.74 (q, 4H, H-49-34), 3.48 (dd, J=4.0,8.0 Hz, 2H, H-35), (dd, J = 12.0, 4.0 Hz, 1H, 18-H), 2.54 (dd, J=4.0,12 Hz, 1H, H-18). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) 176.99 (C27), 152.48 (C37), 150.11 (C39), 145.13 (C41), 144.41 (C12), 130.21 (C43), 122.48 (C42), 121.99 (C13), 118.46 (C46), 113.43 (C45), 112.48 (C44), 78.96 (C2), 70.23 (C34), 68.11(C49), 65.13 (C50). MS (ESI^+) 704.43 calculated for $C_{43}H_{61}ClN_2O_4$ $[M+H]^+$, found 704.11.



Scheme 1. Reagents and conditions: (i) 1,3-diaminopropane 90 ^{0}C, 24 h; (ii) 1,2-diaminopropane 90 ℃, 24 h; (iii) diethelenetriamine 85 ℃, 24 h; (vi) hydrazine 95 ℃, 24 h; (v) ethanolamine 85 ℃, 24 h; (vi) 2(2-Aminoethoxy) ethanol 85 ℃, 24 h.; (vii) oleanolic acid 120 ℃, 24 h.

2.9. Antibacterial Activity

The synthesized hybrid compounds were tested against 11 reference bacterial strains namely: Gram-positive bacteria: *Bacillus subtilis* (ATCC19659), *Enterococcus faecalis* (ATCC13047), *Staphylococcus epidermidis* (ATCC14990), *Staphylococcus aureus*, Gram-negative bacteria: *Enterobacter cloacae* (ATCC13047), *Proteus vulgaris* (ATCC6380), *Klebsiella oxytoca* (ATCC8724), *Proteus vulgaris* (ATCC6380), *Pseudomonas aeruginosa* (ATCC27853), *Proteus mirabilis* (ATCC7002), *Escherischia coli* (ATCC25922) and the mycobacterium, *Mycobaterium smegmatis* (MC2155).

2.9.1. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the synthesized hybrid compounds was assessed using the method as described by Fonkui et al. [48]. Streptomycin, nalidixic acid and OA were used as positive

control against Gram-positive and Gram-negative bacterial strains, respectively. The negative control consisted of 50% nutrient broth in DMSO. Stock solutions were prepared by adding 20 mg of the synthesized compounds to 3 mL of DMSO. The stock solutions were diluted in nutrient broth to obtain the desired concentrations (6.66, 3.33, 1.66, 0.83, 0.42, 0.208, 0.104 mg/mL). Thereafter, 100 µL of each of these solutions was pipetted in duplicate into the wells of a 96-well plate. To this was added 100 µL of bacteria which was diluted to a 0.5 McFarland standard in nutrient broth and the plates were incubated overnight at 37 °C. The analysis was performed in duplicate.

2.10. In vitro cytotoxicity evaluation

Cytotoxicity was measured by determining cell density with the use of the sulforhodamine B (SRB) staining assay on four different cell lines as described by Vichai and Kirtikara [49] with minor modifications. DU 145 (ATCC® HTB-81™), MDA-MB-231 (ATCC® HTB-26™), MCF-7 (ATCC® HTB-22™) and MCF-12A (ATCC® CRL-10782™) cells were used in experiments. DU 145, MCF-7 and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% nonessential amino acids, 1% L-glutamine, penicillin (100 U/mL), streptomycin (100 U/mL) and 10% heat-inactivated foetal calf serum (FCS). MCF-12A cells were cultured in a 1:1 mixture of DMEM and Ham's F12 medium, 20 ng/mL Human epidermal growth factor, 100 ng/mL cholera toxin, 0.01 mg/mL bovine insulin and 500 ng/mL hydrocortisone, 10% heat-inactivated foetal calf serum. Cell cultures were incubated in T75 culture flasks at 37°C in a humidified incubator with a 5% CO_2 atmosphere until confluency was established. Confluent cells (80%) were washed with sterilised phosphate-buffered saline (PBS) and chemically detached with TrypLE™Express dissociation solution. Cells were harvested and centrifuged at 200 g for 5 min; supernatant was discarded and the pelleted cells re-suspended in 1 mL supplemented medium. Cells were counted using the trypan blue exclusion assay (0.1% w/v) and re-suspended to a cell concentration of 5 x 10^4 cells/mL. 100 µL (5 x 10^3 cells/well) of cell suspension was seeded into sterile, clear 96-well flat bottom plates, and incubated overnight to allow for attachment. Blank wells contained 200 µL FCS (10%)-supplemented media without cells to account for background noise and sterility. Attached cells were exposed to 100 µL medium (negative control), experimental compounds (0.001-200 µM) or saponin (1%; positive control) prepared in 10 % FCS supplemented medium for 72 h at 37°C in a humidified incubator with a 5% CO_2 atmosphere. Cells were fixed using 50 µL trichloroacetic acid (50%) overnight at 4 °C. Fixed cells were washed three times with tap water, air dried and stained using 100 µL SRB solution (0.057% in 1% acetic acid) for 30 min. Stained cells were washed four times with 150 µL acetic acid (1%) and air-dried. The bound dye was eluted using 200 µL Tris-buffer (10 mM, pH 10.5) and the absorbance measured a 510 nm (reference 630 nm) using an EL-X 800 microplate reader (Biotek Inc, USA). Compounds were tested in triplicate on each plate on three separate occasions. All values were adjusted by subtracting the blank and the cell density relative to the negative control expressed as a percentage and Graphpad software was used to calculate IC_{50} values.

3. Results and discussion

The 4-aminoquinoline derivatives **3-8** were prepared via amination reaction of selected amines or amino alcohols with 4,7-dichloroquinoline. The compounds **3-8** were obtained in good yields in the range of 62-75%. The compounds contained functional groups suitable for hybridization with OA (**Scheme 1**). The synthesis of 4-aminoquinoline derivatives was carried out via a nucleophilic substitution on the substituted 4.7-dichloroquinoline scaffold [42,43,45,50].

The hybrid compounds **9-12** were prepared by amination reaction of OA with compounds **3-6**. They were obtained in a moderate yield in the range of 51-66%. Their melting point was in the range of 114-133 ºC. Compound **12** displayed the lowest melting point which can be attributed to the short linker between the parent compound, OA and the 4-aminoquinoline derivative, **6** and its low molecular mass when compared to other compounds. FTIR spectra of the compounds **9-12** revealed important bands at 1626-1712 cm^{-1} and at 3296-3549 cm^{-1} for C=O stretch and N-H stretch on the linker, respectively (**Figure 8&9**). ^{1}H-NMR spectra of the compounds **9-12** also displayed signals at 7.02-8.28 ppm for the aromatic protons on the 4-aminoquinoline derivatives and at 177-181 ppm on ^{13}C-NMR spectra for the amide carbon (**Figure 8&9**). The LC-MS displayed peaks for the molecular masses of the compounds which were 673.33, 671.09, 701.23, 631.35 g/mol, respectively for compounds **9**, **10**, **11** and **12** confirming the successful synthesis of the compounds (**Table 1**).

The hybrid compounds **13** and **14** were prepared by esterification reaction of **7** and **8** with OA. These were obtained at a yield of 54 and 51 %, respectively for compound **13** and **14**. Their melting points were in the range of 131-133 ºC. FTIR spectra of the compounds **13** and **14** revealed important bands at 1694-1702 cm^{-1} and at 3450-3535 cm^{-1} for C=O stretch and N-H stretch on the linker, respectively (**Figure 9**). ^{1}H-NMR spectra of the compounds, **13** and **14** also displayed signals at 6.83-7.94 ppm for the aromatic protons on the 4-aminoquinoline derivatives and at 176.99-177.05 ppm on the ^{13}C-NMR spectra for the ester carbon (**Figure 9**). The UHPLC-HRMS displayed peaks for the molecular masses of the compounds which were 660.38 and 704.11 g/mol, respectively for compounds **13** and **14**, confirming the successful synthesis of the compounds (**Figure 9**).

The antibacterial results of hybrid compounds **9-14** and the control, streptomycin and nalidixic acid are provided in **Table 2**. Generally, the antibacterial activity of the hybrid compounds was similar to OA against the strains of bacteria. Compounds **9** and **11-13** were effective in inhibiting *E. faecalis*, *K. oxytoca* and, *E. coli* with MIC value of 1.25 mg/mL, compared to OA with MIC value of 2.5 mg/mL. Compound **14** was selective and effective against *E. faecalis*, and *E. coli* with MIC value of 1.25 mg/mL. Furthermore, compounds **11** and **13** displayed promising antibacterial activity against *S. aureus* when compared to the parent drug, OA. Compounds **9** and **11** inhibited *E. cloacae* and *P. vulgaris* growth with an MIC value of 1.25 mg/mL compared to OA with MIC value of 2.5 mg/mL. The

antibacterial activity of compound **10** was also comparable to OA revealing that the nature of the compound hybridized with OA influenced the antibacterial activity of the compound.

Table 2: Antibacterial activities of synthesized compounds.

	Minimum inhibitory concentration (MIC, mg/mL)										
Tested hybrid compound	**Gram-positive**					**Gram-negative**					
	BS	***EF***	***SE***	***SA***	***MS***	***ECL***	***PV***	***KO***	***PA***	***PM***	***EC***
9	2.5	1.25	1.25	2.5	2.5	2.5	2.5	1.25	2.5	2.5	1.25
10	2.5	2.5	2.5	2.5	2.5	1.25	2.5	2.5	2.5	2.5	2.5
11	2.5	1.25	1.25	1.25	1.25	1.25	1.25	1.25	2.5	1.25	1.25
12	2.5	1.25	1.25	2.5	1.25	2.5	2.5	1.25	2.5	1.25	1.25
13	2.5	1.25	1.25	1.25	1.25	1.25	2.5	1.25	2.5	2.5	2.5
14	2.5	1.25	2.5	2.5	1.25	2.5	2.5	2.5	2.5	1.25	1.25
OA	2.5	2.5	1.25	2.5	1.25	1.25	2.5	2.5	0.078	1.25	2.5
STM (µg/mL)	16	128	8	256	4	512	128	16	16	128	64
NLD (µg/mL)	16	>512	64	64	512	16	128	8	256	32	512

STM: *Streptomycin*, **NLD:** *Nalidixic acid*, **OA:** Oleanolic acid, ***BS:*** *Bacillus subtilis,* ***EF:*** *Enterococcus faecalis,* ***SE:*** *Staphylococcus epidermidis,* ***SA:*** *Staphylococcus aureus*, ***MS:*** *Mycobacterium smegmatis,* ***ECL:*** *Enterobacter cloacae,* ***PV:*** *Proteus vulgaris,* ***KO:*** *Klebsiella oxytoca,* ***PA:*** *Pseudomonas aeruginosa, and* ***EC:*** *Escherichia coli.*

The antibacterial studies of OA on *E. coli* have been reported to affect the efflux pumps thereby interfering with the viability of the bacteria [51]. Its mode of action on bacteria is also via the induction of a stress response [52]. It also acts by inhibiting the turnover of peptidoglycan in bacteria and causing destruction of the cellular wall of bacteria [53]. Oleanolic acid possesses a wide range of antibacterial activity, which reveals that it is a promising precursor for the development of drugs for the treatment of drug-resistant bacteria species.

Cellular iron plays an important role in cell composition, enzyme activity metabolism etc. It is not soluble at a neutral pH and its uptake by the phagocyte is influenced by an acidic environment in the

endocytic lysosomes and vesicles. 4-Aminoquinoline such as chloroquine increases lysosomal and endocytic pH of the cells which interferes with normal iron metabolism thereby inhibiting the intracellular multiplication of bacteria [54]. 4-Aminoquinoline derivatives disrupt the microbial cell membrane thereby increasing the permeability of the membrane. They also act as inhibitors of antibiotic efflux pumps in multidrug resistant microorganisms [55], by interacting with the bacterial respiratory chain [56].

In vitro cytotoxicity evaluation on selected cancer cell lines namely: MDA, MCF-7, MCF-12A, DU145 revealed that compounds **13** and **14** were more cytotoxic to MCF-7 and DU145 cells than OA (**Table 2)**. Compound **14** was more cytotoxic in both cell lines when compared to compound **13**, suggesting that the ester functional group and the length of the linker played a significant role in their cytotoxic activity on the cancer cell lines. Compound **13** was not toxic to the normal cell lines whereas compound **14** exhibited moderate toxicity in MCF-12A cells. Compounds **9-14** did not display any significant cytotoxic effect towards MDA cell lines. Compounds **9-12** which contain amide linker and short linkers between both scaffolds (oleanolic acid and 4-aminoquinoline) did not display any cytotoxic activity in the cell lines tested against. The aforementioned findings suggest that shorter linker resulted in compounds with rigid structures which nullify the biological activity of the compounds. Compounds **13** and **14** with longer linkers displayed promising anticancer activity compared to OA. Some researchers have reported oleanolic acid-based hybrid compound with good anticancer activity [57–59]. The mode of action of oleanolic acid-based derivatives against cancer cell lines has been reported to be via mechanisms such as induction of cell cycle arrest, by activating apoptosis and autophagy pathways [60,61]. Although OA has been reported to exhibit anticancer activity, it can be further enhanced via hybridization with other known scaffolds with anticancer activity. Hybridization is a promising approach useful for the development of potent compounds.

Table 3: *In vitro* cytotoxicity of compounds **9-14**

Compounds	IC_{50} (µM)		
	MCF-7	**MCF-12A**	**DU145**
9	> 200	> 200	> 200
10	> 200	> 200	> 200
11	> 200	> 200	> 200
13 VK7	147 ± 1.08	> 200	153.6 ± 1.07
14 VK8	97.5 ± 1.07	96.87 ± 1.03	88.09 ± 1.02
Oleanolic acid (OA)	> 200	> 200	> 200

4. Conclusions

The derivatives, **9-14** were synthesized and obtained in moderate yield in the range of 51-66%. FTIR, NMR and LC-MS analysis confirmed the successful synthesis of the compounds. Some of the hybrid compounds inhibited the growth of *E. faecalis, S. aureus*, *P. vulgaris, K. oxytoca,* and *E. coli*. Furthermore, the hybrid compounds **13** and **14** with ester linkers, displayed promising cytotoxic activity in MCF-7 and DU145 cell lines, with a mild toxic effect on normal MCF-12A breast cell line when compared to compounds **9-11** which contain amide linkers and displayed no significant cytotoxic effect *in vitro*. The present study revealed that modification of C28 of OA can result in the enhancement of its biological properties. Furthermore, hybridization of OA with selected pharamcophores is a potential approach to develop compounds with good biological activity.

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