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RESEARCH ARTICLE

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Synthesis of Ester-Linked Ursolic Acid-Based Hybrid Compounds: Potential Antibacterial and Anticancer Agents

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The molecular hybridization of two or more drugs into a single molecule is an effective drug design approach to reduce pill burden and improve patient treatment adherence. Ursolic acid-based hybrid compounds were synthesized and characterized followed by molecular docking studies. *In vitro* studies against various bacterial strains and human cancer cells (MDA-MB-231, HeLa, and MCF-7) were performed. Compounds **14**–**19**, **21**, **34**, **31**, and **30** demonstrated significant antibacterial activities with MIC values of 15.625 μg/ml. Compounds **29** and **34** were more cytotoxic than ursolic acid, with IC_{50} values of 46.99 and 48.18 μg/ml. Compounds **29** and **34** in the docking studies presented favourable binding interactions and better docking energy against the Epidermal Growth Factor Receptor (EGFR) than the parent compound, ursolic acid. The findings revealed that the ursolic acid scaffold is a promising precursor for the development of molecules with promising anticancer and antimicrobial activities. However, more studies are needed to fully understand their mode of action.

Keywords: Ursolic acid, Hybrid molecules, Antibacterial, Anticancer, Molecular Docking.

Introduction

The search for novel pentacyclic triterpenoid derivatives with potency for numerous biological targets continues to be a fascinating scientific endeavour. It is well recognized that the structural modification of active natural compounds is a successful method for developing novel therapeutic drugs. The rate of use of natural medicine to treat infectious diseases has increased to 75%.[1] Among the class of pentacyclic triterpenoids, ursolic acid, **1** (*Figure 1*) is a privileged structural motif found in many natural sources that have gained a lot of attention in recent years due to its various pharmacological potential, including anticancer and antibacterial properties etc.[2,3] The development of new therapeutic drugs whereby the structures are inspired by natural compounds is a promising approach for addressing the problem of multidrug-resistant bacteria (MDR). Previous studies have shown that when natural products are hybridized with other pharmaceutical scaffolds, they become more efficient.[4] Hybridization of pharmacophores where two or more bioactive moieties are covalently linked and available as a single hybrid entity has emerged as a preferred drug discovery technique for

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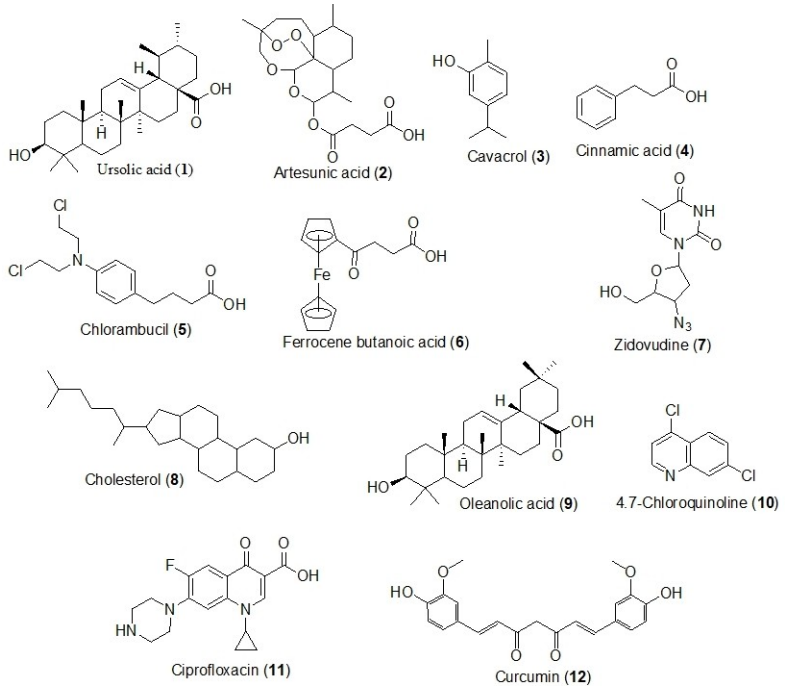

Figure 1. Structures of known pharmaceutical scaffolds hybridized with ursolic acid.

the synthesis of novel compounds with distinct biological activities.[5] Ursolic acid has some drawbacks, including a short half-life, poor solubility, and low absorption, resulting in minimal effectiveness against cancer cells or bacterial strains, making it ineffective.[6,7] Hybridizing ursolic acid with other pharmaceutical scaffolds to prepare new compounds with better anticancer and antibacterial activity profiles has been discovered.[8,9,10] Ursolic acid derivatives have been reported to inhibit a variety of cancer pathways, including aromatase inhibition, kinase inhibition, cell cycle arrest, angiogenesis inhibition, heat shock protein (HSP90) inhibition, telomerase inhibition, antimitotic activity, and sulfatase inhibition[11–15] A hybrid molecule is said to possess not just the properties of its parent compounds. but also new or improved properties as a result of the synergistic interaction of drug molecules. Although several ursolic acid hybrids have been developed, only a few have been tested for antibacterial and anticancer properties via modification of the carboxylic functional group on ursolic acid. Ursolic acid arylidene-hydrazide hybrids prepared from hydrazide and using selected aromatic aldehydes displayed promising antibacterial activity against *E. coli*, *C. Albicans*, and *S. aureus*.[16] Piperazine-tailored ursolic acid hybrids were effective in inhibiting the growth of *X. axonopodis* pv. *citri.* and *Xanthomonas oryzae* pv. *Oryzae*.[17] Ursolic acid-thymine hybrids displayed moderate antiproliferative activity *in vitro*.[18] The cycloaddition of azidopropyl-3β-hydroxy-urs-12-en-28-oate with C28 propargyl esters of ursolic acid via Click reaction conditions produced hybrid molecules with potent inhibitory activities against the breast cancer cell lines, MDA-MB-231.[19]

In this study, the pharmaceutical scaffolds **1**–**12** in *Figure 1* were chosen for their efficient therapeutic activities. The compounds shown in *Figure 1* have been widely reported to exhibit antibacterial and anticancer activities. However, to further enhance their therapeutic efficacy, they were hybridized with ursolic acid to form hybrid compounds. Ursolic acid is characterized by low toxicity, but its poor water solubility results in its poor bioavailability which hinders its inability to induce significant cytotoxic effects.[20,21] To enhance its bioavailability and to exploit its low toxicity, it was hybridized with known pharmaceutical agents shown in *Figure 1*. For decades, artesunate, derived from the traditional Chinese medicinal plant (*Artemisia annua* L.,) is known as the first-line treatment for malaria due to its endoperoxide group. Various artemisinin derivatives have been developed in recent years to enhance its therapeutic potential such as antiviral, anticancer and anti-inflammation. Among these, artesunate has received the most attention due to its high pharmacokinetic and clinical value attributed to a hemisuccinate group,

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which increases its water-solubility and oral bioavailability.[22] Carvacrol has received attention in recent years for its antibacterial activities as well as additional biological activities, such as anticancer, anxiolytic, antidepressant and antifungal activities. Additionally, due to its inclusion on the European Commission's list of chemical flavourings and approval by the Food and Drug Administration (FDA) as a toxicologically safe compound and it is used as an additive in food products.[23] Cinnamic acid, derived from plants such as whole grains, *Panax ginseng*, *Cinnamomum cassia* (Chinese cinnamon), fruits, and vegetables, is low in toxicity and has a wide range of biological activities. Cinnamic acid-based derivatives are promising compounds that have a high potential for drug development. Many cinnamic acid derivatives, particularly those with the phenolic hydroxy group, are well-known antioxidants with numerous health benefits due to their powerful free radical scavenging properties.[24] Chlorambucil is a nitrogen mustard alkylating agent that is used as a cytostatic drug in cancer therapy.[25] Ferrocene is one of the well-known organometallic compounds. Ferrocene derivatives have been linked to a variety of biological activities, such as antitumor, antimalarial, antioxidant, analgesic, anti-HIV, antineoplastic, anticonvulsant, antimicrobial, and DNA cleaving properties. The antibacterial, antitumor and antimalarial properties of ferrocene derivatives have attracted a lot of attention.[26] Zidovudine is an FDA-approved AIDS prevention and treatment drug that is also on the list of the World Health Organization's Essential Medicines.[27] Ciprofloxacin, the second generation of fluoroquinolone exhibits excellent antimicrobial activity, pharmacokinetic properties, and few side effects, and has been used in clinical practice for the treatment of various bacterial infections for nearly three decades.[28] The WHO has recommended Ciprofloxacin as a second-line agent for the treatment of tuberculosis (TB), primarily in cases of resistance or intolerance to first-line anti-TB therapy. Ciprofloxacin derivatives have thus sparked an ongoing interest. In the last 30 years, numerous ciprofloxacin derivatives with diverse pharmacological activities such as antibacterial, anti-oxidation, anti-malarial, anti-fungal, anti-HIV, and anticancer activities have been developed, and the antibacterial property remains the domain of research.[29] Curcumin has derived naturally from the plant *Curcuma longa* and has been shown to have potent tumor-suppressor activity in clinical studies. Curcumin's therapeutic effects are primarily due to three different biological activities: it has antioxidant activity (at low concentrations), protein binding capacity, and metal-chelating activity.[30] We incorporated the chloroquine structure (**10**) into the ursolic acid after being inspired by our previously published oleanolic acid-based chloroquine hybrid molecules with promising antibacterial activities.[4] Oleanolic acid is a pentacyclic-triterpenoid and an isomer of ursolic acid and is found in a variety of plant species. It is significant in organic synthesis because it provides a new framework for the semi-synthesis of promising therapeutic agents.

Life-threatening infections caused by MDR microorganisms continue to be a major public health concern around the world. According to recent statistics, an estimated 700,000 deaths occurred worldwide in 2017.[31,32] Furthermore, experts predict that by 2050, an estimated 10 million people will die each year because of infections caused by pathogenic-resistant bacteria.[33] The African region had higher rates of prevalence, mortality, and morbidity when compared to other regions.[34,35] The development of therapeutic drugs with structures inspired by natural products is a promising technique for addressing the issue of MDR.[36–40] The synthesis of eleven ursolic acid-based hybrid compounds, and their spectroscopic properties such as FT-IR, LC/MS-MS, ^{1}H and ^{13}C-NMR spectroscopic methods, are reported in this article. Potential antibacterial activities of synthesized hybrid compounds were tested against eleven bacterial strains ($Gram^{+}$ and $Gram^{-}$) using microdilution methods: $Gram^{+}$ bacteria included *B subtilis* (**BS**) (ATCC19659), *Enterococcus faecalis* (**EF**) (ATCC13047), *Mycobacterium smegmatis* (**MS**) (MC2155), *Staphylococcus epidermidis* (**SE**) *and S aureus* (**SA**) (ATCC25923). $Gram^{-}$ bacteria included *Enterobacter cloacae* (**ECL**) (ATCC13047), *Proteus vulgaris* (**PV**) (ATCC6380) *Escherichia coli* (**EC**) (ATCC25922), *Klebsiella oxytoca* (**KO**) (ATCC8724), *Proteus mirabilis* (**PM**) (ATCC7002) *and Pseudomonas aeruginosa* (**PA**) (ATCC27853), and *Pseudomonas aeruginosa* (**PA**) (ATCC 2785). In addition, the potential cytotoxicity of the synthesized hybrid compounds was investigated using the MTT assay in MCF-7, MD-MBA-231, and HeLa cells.

Results and Discussion

In this study, hybrid compounds consisting of various pharmaceutical scaffolds such as ursolic acid (**1**), artesunate (**2**), carvacrol (**3**), cinnamic acid (**4**), chlorambucil (**5**), ferrocene butanoic acid (**6**), zidovudine (**7**), cholesterol (**8**), oleanolic acid (**9**), 4,7-Dichloroquinoline (**10**) ciprofloxacin (**11**), curcumin (**12**), have

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been designed and synthesized. In total, seventeen hybrid compounds (**13**–**27**) were synthesized as shown in *Scheme 1*. All of the pharmaceutical scaffolds in *Figure 1* were purchased commercially and used without further modification. The ursolic acid-based hybrids were synthesized according to the procedures outlined in previous studies.[41,4]

The structure-activity relationship of the synthesized hybrid compounds was evaluated based on their *in vitro* activity against bacterial strains and cancer cell lines. Mono-esterified ursolic acid-based hybrid molecules (**13**–**19**) were synthesized in a one-step esterification reaction and yielded 52–66%. The reaction mixture required 4–5 days of stirring. To synthesize hybrid compounds **21**, **23**, and **25**, succinic anhydride was used as a linker compound. Ester bonds were formed between the free alcohol groups of zidovudine, cholesterol, and oleanolic acid. The obtained derivatives **20**, **22**, and **24** containing the carboxylic groups were then linked to the free alcohol group of ursolic acid via esterification reaction yielding 78%, 80% and 55%, respectively.

To synthesize intermediate **24**, oleanolic acid was reacted with 3-(diethylamino)propylamine *via* amidation reaction and the reaction was stirred overnight at 120 °C. Following that, intermediate **24** was reacted with ursolic acid in the presence of HSU and DCC to produce the target hybrid compound **25**. 2-Chloroacetyl chloride was reacted to ciprofloxacin in tetrahydrofuran (THF) in the presence of triethylamine for 1 h at room temperature, and the resulting intermediate **28** was crystallized from acetonitrile. Intermediate **28** was then treated with ursolic acid and the resulting product (hybrid **29**) yielded 58%. Di-esterified ursolic acid-based carvacrol hybrids **30**, **31**, and **32** were obtained in 66%, 52% and 55% yields, respectively. Lastly, the 4,7-dichloroquinoline scaffold was nucleophilically substituted with amino alcohols to yield two intermediate compounds **33** and **35** in 78% and 88% yields, respectively. Both compounds **33** and **35** were treated with ursolic acid on anhydrous DMF in the presence of DCC and DMAP to hybrid **34** and **36** in **62** and **54%** yield, respectively.

Antibacterial Activity

As shown in *Table 1*, the disc diffusion method revealed that ten tested hybrid compounds had moderate to strong activity against both Gram-positive and Gram-negative organisms. Among the tested compounds, hybrid compounds **14**–**19**, **21**, **34**, **31**, and **30** had a more potent antibacterial effect on some bacterial strains. Hybrid compounds **13**, **14**, **15**, **34**, **29**, **25** and **30** showed more potent activity against both Gram-positive and Gram-negative. Compounds **13** and **14** showed more potent activity against PV, compound **15** showed high activity against BS, EF, SA, PV, KO, PM, and EC. In addition, only compound **29** displayed a significant antibacterial effect against all the tested bacterial strains, revealing a synergistic effect resulting from the hybridization of ciprofloxacin and ursolic acid. However, compound **25** showed high activity against BS, EF, PV and EC. Also, compound **30** indicated remarkably high activity against two Gram-

Table 1. Minimum inhibitory concentration (MIC, μg/mL) of the hybrid compounds.

Compound	Minimum inhibitory concentration (MIC, μg/mL)										
	Gram-positive					*Gram*-negative					
	BS	***EF***	***SE***	***SA***	***MS***	***ECL***	***PV***	***KO***	***PA***	***PM***	***EC***
13	250	250	500	250	500	500	15.625	500	250	125	250
14	125	250	500	31.25	250	250	15.625	250	250	62.5	250
15	15.625	15.625	31.25	15.625	62.5	31.25	15.625	15.625	250	15.625	15.625
16	62.5	31.25	125	62.5	125	125	62.5	125	62.5	15.625	62.5
17	62.5	62.5	250	62.5	250	250	62.5	125	250	62.5	62.5
18	31.25	125	250	125	250	250	125	125	250	125	62.5
19	62.5	125	250	125	125	125	125	125	250	62.5	125
21	62.5	31.25	125	62.5	125	125	31.25	125	125	31.25	62.5
23	125	125	250	125	250	250	125	250	250	125	125
25	15.625	15.625	125	31.25	125	62.5	15.625	62.5	250	31.25	15.625
27	125	125	250	125	250	250	125	250	250	250	125
29	15.625	15.625	15.625	15.625	15.625	15.625	15.625	15.625	15.625	15.625	15.625
31	62.5	62.5	250	62.5	125	125	62.5	250	250	62.5	62.5
32	125	125	125	250	250	250	125	250	250	125	125
30	31.25	31.25	250	31.25	250	125	15.625	250	250	15.625	31.25
Ursolic acud	15.625	31.2	125	31.25	125	125	15.625	31.25	250	15.625	31.25

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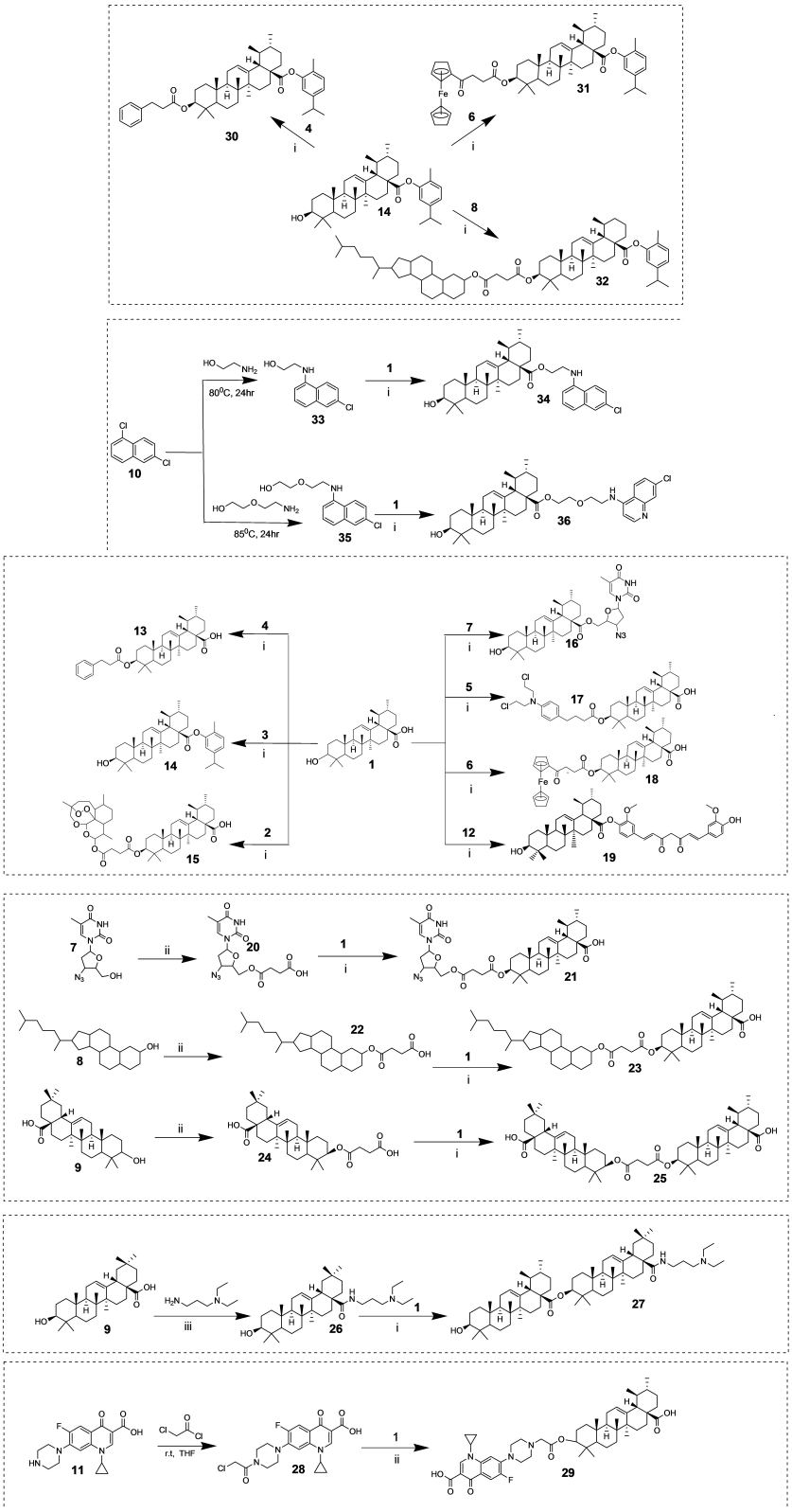

Scheme 1. Synthetic routes of ursolic acid-based hybrids **13**–**36**; i) DCC, DMAP, DMF, 0 °C→r.t., (ii) succinic anhydride, DMAP, Pyridine, r.t. Iii DMF, DCC, HSU 80–85 °C.

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negative bacterial strains such as PV and PM. The hybridization of three molecules, cinnamic acid, ursolic acid, and carvacrol, resulted in improved antibacterial activity when compared to the hybridization of ursolic acid and carvacrol which revealed an antagonistic effect.

Compound **13** containing cinnamic acid was found to be less active than both parent compounds (UA and Cinnamic acid) against all the tested bacterial strains, except in PV where it indicated a MIC value of 15.625 μmol/mL which is similar to that of UA. This indicates that linking cinnamic acid to UA at C3 reduces its anti-bacterial activity, suggesting an antagonistic effect. Similar antagonistic effects have been reported when combining a derivative of cinnamic acid with selected antimicrobial agents.[42] Compound **14** also demonstrated lower antibacterial activity than its parent compounds against the majority of bacterial strains and it only showed synergistic effects against SA, PV and PM with MIC values of 31.25, 15.625, and 62. 5 μg/mL, respectively. The reduced antibacterial efficacy of compound **14** is attributed to the modification of the carvacrol via the hydroxy functional group. The hydroxy group on carvacrol play an important role in its antibacterial activity and modifying the hydroxy group, decreases the antibacterial activity of the hybrid molecules.[43–45] The antibacterial studies revealed the efficacy of ursolic acid-based hybrid compounds as potential antibacterial agents. However, some of the hybrid molecules displayed selective antibacterial activity against some strains of bacteria. There is still a need to fully understand the mode of action of these compounds.

In vitro *Cytotoxicity Evaluation*

The *in vitro* cytotoxic activities of the synthesized ester-linked ursolic acid-based hybrid compounds (**19**, **29**–**32**) on human cancer cell lines (MCF-7, MD-MBA-231 and HeLa) were determined using an MTT test. In this study, selected cancer cell lines were used. MCF-7 is often used to study estrogen receptor-positive breast cancers.[46] It is not aggressive and is also a non-invasive cell line with low metastatic potential.[47] On the other hand, MDA-MB-231 cell lines are aggressive and invasive with high metastatic potential.[48] HeLa cell lines have been generally classified as cervical cancer cells. However, studies have shown that HeLa and MDA-MB-648, breast cancer cell lines have common G6PDH mobility of A-type and isoenzyme MGM1. It is aggressive and metastatic.[49]

Table 2 shows the cytotoxic activity values of these hybrids expressed as IC_{50}. Hybrids **29** and **34** demonstrated interesting cytotoxic activities against MCF-7 cells, with IC_{50} values of 46.99 and 48.18 μg/ml, respectively. Compounds **29** and **34** were composed of ursolic acid with ciprofloxacin, and 4-aminoquinoline derivative, respectively. Ciprofloxacin hybrids have been reported to exhibit anticancer activity.[50,51] Ciprofloxacin's anticancer effect results from its ability to hinder the topoisomerase II enzyme, a know cellular target for anticancer drugs, such as doxorubicin. It also promotes an intrinsic apoptotic pathway.[50,51] Ursolic acid exhibit anticancer activity by inhibiting cell proliferation via different signalling pathways including PI3 K/Akt/mTOR-, ERK-, and EGFR. It also induces apoptosis, reduces the tumor size and inhibits tumor growth and metastasis.[52,53] Quinoline derivatives exhibit anticancer activity via different mechanisms, including inhibition of tyrosine kinases, tubulin polymerization, and topoisomerase, together with cell cycle arrest in the G2 phase.[54]

Molecular Docking

The molecular docking studies were performed using EGFR protein which is usually overexpressed in most types of cancer. The predicted binding pattern in this study revealed that the newly synthesized compounds exhibited a perfect fitting in the active site of the EGFR (1M17) target, utilizing hydrogen bonds, pi-cation, and salt bridge interactions. In many types of cancer, the overexpression of EGFR has been reported to result in resistance to therapy due to aggressive invasiveness.[55.56] Its overexpression in breast cancer is linked with poor clinical outcomes and large tumor size.[57] It is overexpressed frequently in triple-negative and inflammatory breast cancer which are usually

Table 2. Cytotoxicity of hybrid molecules **1**, **19**, **29**–**32**, **34**, **36** against human cancer cells MCF7, MD-MBA-231 and HeLa cells.

Compound	IC_{50} (μg/ml) MCF-7	MD-MBA-231	HeLa
1	49.06 ± 0.15	NT	49.64 ± 0.11
19	NT	73.02 ± 0.0793	NT
29	46.99 ± 0.11	NT	55.21 ± 0.17
30	54.89 ± 0.13	NT	59.98 ± 0.18
31	55.60 ± 0.13	58.47 ± 0.06	64.75 ± 0.19
32	51.06 ± 0.12	NT	61.52 ± 0.18
34	48.18 ± 0.11	NT	104.69 ± 0.32
36	NT	85.64 ± 0.09	NT

* NT means not tested.

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aggressive.[57,58] Several known anticancer drugs have been investigated as potential EGFR inhibitors.[59] The mechanisms of EGFR overexpression are *via* EGFR gene amplification. activating mutations of EGFR, *etc*.[57] EGFR is reported to induce cancer cell migration and invasion. EGFR overexpression is found in over 50% of cases of triple-negative breast cancer,[60] making EGFR inhibitors promising targeted agents for the treatment of triple-negative breast cancer. Studies have shown that EGFR is an effective target for the treatment of human cancer.[61] Ursolic acid has also been reported to inhibit the expression of EGFR.[62] Based on the above-mentioned findings and the efficacy of EGFR as a therapeutic target for the treatment of cancer, we selected EGFR for docking studies.

As shown in *Table 3*, the interactions of compounds **29** and **34** revealed docking scores of −5.08 and −4.70 kcal/mol, respectively. The parent compound, ursolic acid had the lowest docking score (−1.97 kcal/mol) while the co-ligand, 4-anilinoquinazoline interact with the target with a docking score of −7.13 kcal/mol. The glide Emodel scores also showed a similar trend establishing the values −89.61, −68.56, −42.11 and −46.53 kcal/mol for the compounds 29, 34, ursolic acid and the co-ligand, respectively (*Table 3*). Emodel was developed for pose selection and it uses the Glide score to rank poses against one another, where a low value indicates good protein-ligand binding affinity.[63,64] Therefore, the result from this study indicates that the hybrid compounds established more energetically favourable interactions than ursolic acid in the active site of the protein.

Table 3. The binding energy (kcal/mol) and molecular interactions of the docked compounds against EGFR protein.

Compound	Docking score	Emodel score	Amino acids-Ligand interactions
29	−5.08	−89.61	H-bonds (Met 769:O, Asp 831: N^+H) Pi-cation (Phe 699: N^+H) Salt bridge (Asp 831: N^+H)
34	−4.70	−68.56	H-bonds (Asp 831: HN, Lys 851: OH) Halogen bond (Met 769: Cl)
Co-ligand	−7.13	−46.53	H-bond (Met 769: N)
Ursolic acid	−1.97	−42.11	H-bonds (Lys 721: O, Asp 776: OH) Salt bridge (Lys 721: O^-)

As illustrated in *Figure 2*, each of the amide and carboxy moieties of compound **29** was involved in hydrogen bond interactions with the residues, Met 769 and Asp 831 of the target protein. The compound also bonds with Phe 699 and Asp 831 through one pi-cation and salt bridge interactions, respectively. The amide and hydroxy group on compound **34** interacts with the target protein at Asp 831 and Lys 851, respectively, resulting in two hydrogen bond formations. In addition, the chlorine atom from compound **34** formed a halogen bonding interaction with the Met 769 from the protein. Similarly, Met 769 forms a hydrogen bond interaction with one of the nitrogen atoms present in the co-crystallized ligand. In the binding model of the parent compound ursolic acid, the carboxylic moieties exhibited a hydrogen bond and salt bridge interaction with the Lys 721, and interaction between the hydroxy group and Asp 776 residue resulted in the formation of a hydrogen bond. Overall, the docking studies conclude that the hybrids have a good binding affinity for the protein, EGFR as compared to the parent compound.

Conclusions

In conclusion, a series of ursolic acid-based hybrid compounds were designed, synthesized, and tested for *in vitro* antibacterial and cytotoxic activities. When compared to the positive controls, some of the hybrid compounds demonstrated promising antibacterial and cytotoxic activities. Among them, compound **29** with ciprofloxacin moiety demonstrated significant antibacterial activity against all bacterial strains tested. Furthermore, the ciprofloxacin scaffold demonstrated antibacterial activity comparable to compound **29**. The antibacterial activity of these hybrid compounds was closely related to their structural properties. When compared to ursolic acid, hybrids **29** and **34** demonstrated improved cytotoxic activities against MF-7 cells, with IC_{50} values of 46.99 and 48.18 g/ml, respectively. The docking studies reinforced that the hybrid compounds showed a higher binding affinity for the MCF-7 protein target as compared to the parent compound ursolic acid. Therefore, compounds 29 and 34 could be promising candidates for further drug discovery research against human cancer. Overall, these findings could pave the way for the future development of this class of ursolic acid-based hybrid compounds as antibacterial or anticancer agents.

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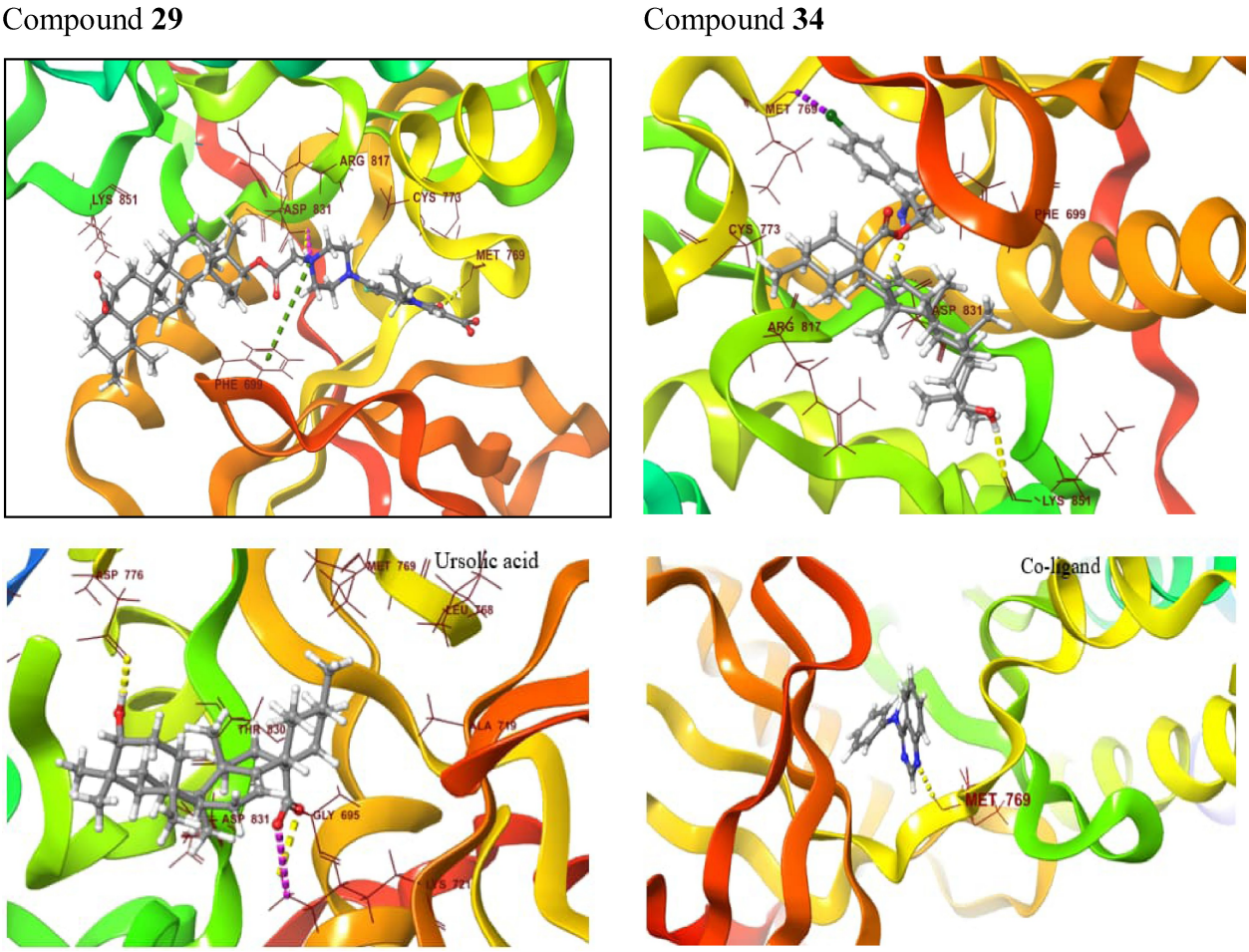

Figure 2. Binding modes and interactions of the docked compounds in the active site region of EGFR (1 M17) protein. The amino acid residues are represented as a three-letter code, hydrogen bonds are represented in yellow dotted lines, salt bridge interactions are shown in magenta lines, pi-cation are in green, and purple dotted lines represent halogen bonds. The compounds are found at the center of each complex and are represented by stick models.

Experimental Section

All reactions were carried out under a nitrogen atmosphere in flame-dried glassware. All the successfully synthesized hybrid compounds were precipitated in a Dichloromethane/hexane mixture after column chromatography to provide a pure product for spectroscopic analysis and biological assays. All the organic solvents used were purchased in HPLC grade and DMF was dried initially over molecular sieves before use. The reagents used in this study were obtained from Sigma–Aldrich (Johannesburg, South Africa) and used without further purification. The compounds were purified by column chromatography using silica gel with a particle size of 40–63 μm, a pore size of 60 Å and a mesh particle size of 230–400. All fractions were collected and visualized on a Thin Layer Chromatography (TLC) plate using a UV light and Vanillin/sulfuric acid (in 95% EtOH) solution. The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Nuclear Magnetic Resonance Spectrometer 500 MHz at room temperature using deuterated Dimethyl sulfoxide ((D_6)DMSO). An Ultra High-Pressure Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HR-MS) spectrometer (Kyoto, Japan) was used to record the high-resolution mass spectra.

General Procedure for the Synthesis of Hybrids

Ursolic acid and the appropriate pharmaceutical scaffolds were dissolved in anhydrous DMF in a dried round bottom flask under a nitrogen atmosphere. DMAP and DCC were added at 0 °C and the reaction mixture was stirred continuously at room temperature for 4–5 days (for the synthesis of hybrids **13**–**19**)

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(*Scheme 1a&b*). To synthesize hybrids **21**, **23** and **25**, zidovudine (**7**), cholesterol (**8**) and oleanolic acid (**9**) were reacted with succinic anhydride on their using pyridine, the reaction mixture was stirred continuously at room temperature overnight then the obtained derivatives (**20**, **22** and **24**) were reacted with ursolic acid *via* an esterification reaction. DMAP and DCC were used at 0 °C and the reaction mixture was stirred continuously at room temperature for 5 days to yield hybrids **21**, **23** and **25**. To synthesize hybrid **27**, oleanolic acid was first reacted with 3-(diethylamino)propylamine on anhydrous DMF at 85 °C using DCC and DMAP following a procedure by Kahnt et al. 2018[65] then the obtained oleanolic acid derivative(**26**) was reacted with ursolic acid at 0 °C using DCC and DMAP, the reaction mixture was stirred continuously at room temperature for 4 days. For the synthesis of hybrids **34** and **36**, chloroquine derivatives (**33** and **35**) synthesized by using our previously published protocol were reacted to ursolic acid using DMAP and DCC at 0 °C and the reaction mixture was stirred continuously at room temperature for 7 days.[4] For the synthesis of hybrid **29**, ciprofloxacin was first reacted with chloroacetyl chloride using THF and the reaction was stirred overnight at room temperature following a published procedure by Qandil et al. 2014.[66] The obtained product (**28**) was then mixed with ursolic acid in DMF using DCC and DMAP at room temperature for 4 days. Hybrids **31**, **32**, and **33** were synthesized by reacting the ursolic acid-based carvacrol hybrid (**14**) with ferrocene-4-ketobutanoic acid (**6**), cholesterol (**8**) and cinnamic acid (**4**), respectively, using a similar procedure to that of hybrids **13**–**19**. TLC, UV light, and a Vanillin/sulfuric acid (in 95% EtOH) spray reagent were used to monitor the reaction. To obtain pure hybrids, the crude product was purified using column chromatography with various eluent solvent systems such as CH_2Cl_2/MeOH, CH_2Cl_2/AcOEt, MeOH/Triethanolamine, (TEA)/Hexane Hexanes/AcOEt.

Hybrid 13: Ursolic acid (**1**) (100 mg, 1.09 mmol, 1.0 equiv.), Cinnamic acid (**4**) (161.49 mg, 1.09 mmol, 1.0 equiv.), DMF (15 mL), DMAP (133.17 mg, 1.09 mmol, 3.0 equiv.), DCC (247.39 mg, 1.2 mmol, 1.1 equiv.). Column conditions: Hexanes/AcOEt 3:7 R_f = 0.5, m.p. 121–124 °C, white powder, Yield: 66%. IR (ATR, γ cm^{-1}): 3307 cm^{-1} (OH), 2933–2898 cm^{-1} (CH aliphatic), 1556 cm^{-1} (C=O), 1462 cm^{-1}(C=C alkene), 1378 cm^{-1} (C=C aromatic). ^{1}H-NMR (500 MHz, DMSO): δ (ppm) 12.29(s, 1H, H-43), 7.67–7.66(m, 1H, H-2), 76.0–7.57(m, 2H, H-4&H-6), 7.40–7.39(m, 2H, H-1&H-3), 6.54–6.30(dd, *J* = 4.0 1H, H-37), 3.00–2.97(dd, *J* = 8.0, 1H, H-12), 2.11-2.08(t, *J* = 4.0, 2H, H-7), ^{13}C-NMR (500 MHz, (D_6)DMSO): δ (ppm) 178.28(C11), 167.56(C9), 143.88(C36), 138.18(C5), 134.24(C1), 130.18(C3), 128.88(C6), 128.17(C4), 124.57(C2), 119.26(C37), 76.84(C12), 54.78(C18), 52.37(C34), 47.01(C27), 46.82(C39), 41.63(23), 39.85(C21), 39.18(C32), 39.09(C14), 39.01(C15), 38.49(C30), 38.44(C29), 38.37(C40), 38.22(C8), 36.22(C20), 32.69(C29), 30.18(C7), 28.25(C25), 27.53(C24), 26.99(C26), 23.81(C38), 23.25(C13), 22.84(C17), 21.06(C16), 17.99(C19), 17.00(C19), 16.07(C33), 15.21(C22). LC/MS ESI$^+$: *m/z* calc.: 588.4179 found: 588.3278 $[M-H]^+$

Hybrid 14: Ursolic acid (**1**) (1000 mg, 2.19 mmol, 1.0 equiv.), Carvacrol (**3**) (328.97 mg, 2.19 mmol, 1.0 equiv.), DMF (10 mL), DMAP (267.55 mg, 2.19 mmol, 2.19 equiv.), DCC (4.97.05 mg, 2.409 mmol, 1.1 equiv.). Column conditions: Hexanes/AcOEt 3:7 R_f = 0.67, white powder, m.p. 118–121 °C, Yield: 52%. IR (ATR, γ cm^{-1}): 3381 cm^{-1} (OH), 2935–2899 cm^{-1} (CH aliphatic), 1802 cm^{-1} (C=O), 1465 cm^{-1}(C=C alkene), 1446 cm^{-1} (C=C aromatic). ^{1}H-NMR (500 MHz, DMSO): δ (ppm) 7.42(d, *J* = 8.0, 1H, H-40), 7.37(d, *J* = 8.0, 1H, H-39), 7.26(s, 1H, H-37), 5.40(t, *J* = 4.0, 1H, H-28), 4.32-4.31(d, *J* = 8.0, 1H, H-2), 3.34(s, 1H, H-43), 3.01(dd, *J* = 4.0, 1H, H-26), ^{13}C-NMR (500 MHz, $CDCl_3$): δ (ppm) 172.31(C2), 157.09(C36), 148.38(C38), 130.13(C40), 129.51(C41), 125.96(C28), 124.00(C39), 120.21(C37), 77.29(C3), 55.22(C9), 52.41(C25), 49.65(C18), 47.98(C30), 47.35(C14), 42.13(C12), 39.08(C23), 38.85(C6), 38.66(C21), 36.95(C5), 35.34(C31), 33.81(C19), 32.99(C43), 31.62(C11), 30.31(C20), 29.45(C16), 28.70(C4), 27.59(C17), 25.80(C15), 24.92(C29), 24.92(C8), 23.26(C7), 23.26(C44), 21.24(C10), 18.39(C22), 17.46(C24), 17.37(C13), 16.52(C32), 15.50(C42). LC/MS ESI$^+$: *m/z* calc.: 588.4154 found: 589.4337 $[M-H]^+$

Hybrid 15: Ursolic acid (**1**) (200 mg, 0.4 mmol, 1.0 equiv.), artesunate (**2**) (153.8 mg, 0.4 mmol, 1.0 equiv.), DMF (10 mL), DMAP (48.9 mg, 0.4 mmol, 1.0 equiv.), DCC (82.5 mg, 0.4 mmol, 1.1 equiv.). Column conditions: CH_2Cl_2/MeOH 9:1. R_f = 0.73, m.p. 106–109 °C, white powder, Yield: 60%. IR (ATR, γ cm^{-1}): 3342 cm^{-1} (OH) carboxylic, 2935–2897 cm^{-1} (CH aliphatic), 1802 cm^{-1} (C=O), 1466 cm^{-1}(C=C alkene), 1457 cm^{-1} (C=C aromatic). ^{1}H-NMR (400 MHz, CDCl3): δ (ppm) 12.04(s, 1H, H-33), 5.57(s, 1H, H-47) 5.21-5.14(d, 1H, H-43), 4.35-4.29(dd, 1H, H-27), 3.46-3.33(dd, 2H, H-54), 3.02(s, 2H, H-54), 2.52-2.52(t, 2H, H-37 & H-38). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm)

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178.24(C1), 173.21(C36), 170.98(C39), 138.17(C26), 124.56(C27), 104.56(C43), 91.71(C46), 90.58(C2), 76.81(C54), 56.00(C8), 54.77(C24), 52.35 (C52), 51.12(C17), 47.00(C29), 46.80(C13), 44.57(C11), 41.62(C22), 39.08(C4), 38.48(C20), 38.42(C18), 38.35(C10), 38.32(C51), 36.51(C19), 36.29(C38), 31.63(C37), 30.17(C15), 28.73(C14), 28.46(C16), 28.23(C28), 27.52(C3), 26.97(C6), 25.50(C7), 23.79(C57), 23.25(C58), 22.83(C58), 21.05(C59), 20.98(C61), 20.03(C9), 18.53(C21), 17.98(C21), 16.99(C23), 16.05(C12), 15.20(C31), 11.50(C62). LC/MS ESI$^+$: *m/z* calc.: 822.5438 found: 823.5320 $[M+H]^+$

Hybrid 16: Ursolic acid (**1**) (200 mg, 0.4 mmol, 1.0 equiv.), zidovudine (**7**) (106.87 mg, 0.4 mmol, 1.0 equiv.), DMF (10 mL), DMAP (48.9 mg, 0.4 mmol, 1.0 equiv.), DCC (82.5 mg, 0.4 mmol, 1.1 equiv.). Column conditions: CH_2Cl_2/EtOH 9:1. $R_f=0.74$, white powder, m.p. 117–119 °C, Yield: 55 %. IR (ATR, γ cm^{-1}): 3360 cm^{-1} (OH) carboxylic, 2937–2899 cm^{-1} (CH aliphatic), 1736 cm^{-1} (C=O), 1470 cm^{-1}(C=C alkene), 1449 cm^{-1} (C=C aromatic). ^{1}H-NMR (400 MHz, CDCl3): δ (ppm) 11.34(s, 1H, H-44), 7.69(s, 1H, H-47), 6.11–6.09 (t, 1H, H-40), 5.23–5.14 (d, 2H,H-36), 4.42–4.39(dd, 1H, H-28), 4.31–4.30(d, 1H, H-37), 3.83-3.81 (m, 1H, H-3), 3.66-3.99(dd,2H, H-29), ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) 178.73(C2), 164.16(C45), 150.86(C43), 138.67(C27), 136.51(C47), 125.05(C28), 109.97(C46), 84.45(C40), 83.88(C37), 77.32(C3), 61.39(C36), 55.25(C9), 52.86(C25), 47.50(C38), 47.31(C30), 42.12(C18), 38.98(C14), 38.92(C6), 38.85(C12), 37.00(C45), 36.79(C46), 36.66(C31), 28.73(C39), 28.02(C11), 23.74(C5), 23.32(C19), 21.53(C19), 21.53(C16), 18.47(C20), 17.47(C4), 17.39(C29), 16.53(C32), 15.69(C15), 12.67(C10). LC/MS ESI$^+$: *m/z* calc.: 705.4465 found: 704.1755 $[M+H]^+$

Hybrid 17: Ursolic acid (**1**) (300 mg, 0.66 mmol, 1.0 equiv.), chlorambucil (**6**) (200 mg, 0.66 mmol, 1.0 equiv.), DMF (10 mL), DMAP (80.63 mg, 0.66 mmol, 1.0 equiv.), DCC (149.79 mg, 0.73 mmol, 1.1 equiv.). Column conditions: CH_2Cl_2/ AcOEt 6:4. $R_f=0.73$, m.p. 114–115 °C, white powder, Yield: 53 %. IR (ATR, γ cm^{-1}): 3424 cm^{-1} (OH) carboxylic, 2932–2893 cm^{-1} (CH aliphatic), 1697 cm^{-1} (C=O), 1460 cm^{-1}(C=C alkene), 1373 cm^{-1} (C=C aromatic). ^{1}H-NMR (400 MHz, CDCl3): δ (ppm) 12.00(s, 1H, H-34), 7.04–7.02(d, 2H, H-48 & H-42), 6.68–6.66(d, 2H, H-45&H-43), 5.50(t, 1H, H-28), 4.31–4.30 (d, 1H, H-3), 3.02-3.00(dd, 1H, H-26), 2.51–2.47(dd, 2H, H-48), 2.51–2.47(dd, 2H, H-49). ^{13}C-NMR (400 MHz, CDCl3): δ (ppm) 172.31(C2), 169.86(C36), 147.02(C44), 140.99(C27), 132.64(C41), 130.31(C42), 130.29(C46), 125.42(C28), 115.25(C45), 115.25 (C43), 77.29(C3), 55.22(C9), 52.41(C25), 49.65(C49), 47.98(C48), 47.35(C18), 42.13(C30), 40.52(C14), 40.45(C50), 40.36(C52), 38.85(C12), 38.66(C23), 36.95(C5), 35.34(C6), 33.81(C21), 32.99(C19), 31.62(C31), 30.31(C40), 29.45(C11), 28.70(C37), 27.59(C20), 25.80(C16), 24.92(C15), 24.08(C17), 23.76(C38), 23.26(C29), 21.39(C4), 18.39(C7), 17.46(C8), 17.37(C10), 16.52(C22), 15.50(C24). LC/MS ESI$^+$: *m/z* calc.: 741.4291 found: 740.4658 $[M-H]^+$

Hybrid 18: Ursolic acid (**1**) (200 mg, 0.4 mmol, 1.0 equiv.), ferrocene-4 ketobutanoic acid (**6**) (132.04 mg, 0.4 mmol, 1.0 equiv.), DMF (10 mL), DMAP (48.9 mg, 0.4 mmol, 1.0 equiv.), DCC (82.5 mg, 0.4 mmol, 1.1 equiv.). Column conditions: Hexanes/ AcOEt 5:1. $R_f=0.73$, m.p. 230–235 °C, brown powder, Yield: 56 %. IR (ATR, γ cm^{-1}): 3434 cm^{-1} (OH) carboxylic, 2942–2895 cm^{-1} (CH aliphatic), 1692 cm^{-1} (C=O), 1462 cm^{-1}(C=C alkene). ^{1}H-NMR (500 MHz, CDCl3): δ (ppm) 12.03(s, 1H, H-54), 5.14(t, 1H, H-28), 4.83 (s, 1H, H-36), 4.57(s, 1H, H-37), 4.29(s, 2H, H-40-44), 3.35(t, 2H, H-49), 3.02(t, 2H, H-50), 2.13–2.11(dd, 1H, H-26). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 180.08(C48), 172.31(C51), 169.86 (C51), 140.99(C27), 125.42(C28), 77.29(C3), 62.35(C38), 55.24(C6), 52.41(C25), 49.65(C18), 47.35(C30), 42.13(C51), 39.65(C14), 38.85(C44), 38.66(C12), 36,95(C23), 35.24(C5), 32.99(C21), 31.62(C22), 30.31(C11), 29.45(C52), 28.70(C20), 27.59(C53), 25.80(C16), 24.81(C17), 24.08(C16), 23.26(C49), 21.24(C4), 18.39(C43). LC/MS ESI$^+$: *m/z* calc.: 760.4729 found: 759.5638$[M+H]^+$

Hybrid 19: Ursolic acid (**1**) (500 mg, 1.09 mmol, 1.0 equiv.), curcumin (**12**) (401.53 mg, 1.09 mmol, 1.0 equiv.), DMF (10 mL), DMAP (133.17 mg, 1.09 mmol, 1.0 equiv.), DCC (247.17 mg, 1.199 mmol, 1.1 equiv.). Column conditions: CH_2Cl_2/AcOEt 7:3. $R_f=$ 0.42, m.p. 210–215 °C, yellow powder, Yield: 58 %. IR (ATR, γ cm^{-1}): 3348 cm^{-1} (OH), 2931–2859 cm^{-1} (CH aliphatic), 1744 cm^{-1} (C=O), 1654 cm^{-1}(C=C alkene), (C=C) 1535 cm^{-1} aromatic. ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 7.69(d, 1H, H-44), 7.64(d, 1H, H-38), 7.23(s, 1H, H-48), 7.21(d, 1H, H-51), 7.22(d, 1H, H-52), 7.06(d, 1H, H-37), 6.86(d, 1H, H-32), 6.80(s, 1H, H-43), 6.73(d, 1H, H-39), 5.50(t,1H, H-13), 4.79(s, 2H,H-41), 3.83(s, 3H,H-54), 3.80(s, 3H, H-57), 3.72(s, 1H, H-55), 3.65(d, 1H, H-18), 3.47(dd, 1H, H-2). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 195.07(C42), 194.57(C40), 174.17(C26), 150.79(C49), 149.62(C33), 148.57(C34), 147.52(C44), 147.52(C38), 145.57(C50), 139.34(C12), 133.40(C45), 127.59(C39), 127.59(C43), 126.92(C36), 124.52(C13),

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123.21(C37), 121.78(C51), 120.61(C52), 115.84(C32), 112.45(C48), 111.85(C35), 76.84(C2), 54.78(C54), 52.37(C57), 47.01(C41), 46.82(C4), 41.63(C18), 40.01(C10), 39.01(C17), 38.49(C11), 38.44(C9), 38.37(C3), 38.22(C22), 36.51(C6), 36.31(C5), 32.69(C21), 30.18(C19), 28.25(C8), 27.53(C20), 26.99(C15), 23.81(C1), 23.25(C28), 22.84(C16), 21.06(C14), 17.99(C27), 17.00(C25), 16.91(C59), 16.07(C7), 16.07(C58), 15.21(C23). LC/MS ESI$^+$: *m/z* calc.: 806.4758 found: 807.5079 $[M+H]^+$

Hybrid 21: Zidovudine (**7**) (500 mg, 1.87 mmol, 1 equiv), succinic anhydride (187.13 mg, 1.87 mmol, 1.0 equiv), pyridine (147.92 mg, 1.87 mmol, 1.0 equiv), DMAP(228.46 mg, 1.87 mmol, 1.0 equiv), DCM(5 ml). Ursolic acid (**1**) (200 mg, 0.4 mmol, 1.0 equiv) Zidovudine derivative (**20**) (147.73 mg, 0.4 mmol, 1.0 equiv), DMAP (48.9 mg, 0.4 mmol, 1.0 equiv), DCC (82.5 mg, 0.4 mmol, 1.1 equiv). Column conditions: Hexanes/AcOEt 7:3. $R_f = 0.39$, m.p. 232–234 °C, white powder, Yield: 50 %. IR (ATR, γ cm^{-1}): 3382 cm^{-1} NH, 3307 cm^{-1} (OH) carboxylic, 2946–2891 cm^{-1} (CH aliphatic), 1686 cm^{-1} (C=O), 1558 cm^{-1}(C=C alkene). ^{1}H-NMR (500 MHz, $DMSO_{-6}$): δ (ppm) 11.94(s, 1H, H-35), 11.34(s, 1H, H-51), 6.10(t, 1H, H-48), 5.23(t, 1H, H-28), 4.42(t, 1H, H-44), 4.31-4.30(d, 2H, H-43), 3.83-3.82(t, 1H, H-2), 3.64-3.61(dd, 2H, H-54), 3.01(t, 1H, H53)^{13}CNMR (500 MHz, CDCl3): δ (ppm) 178.24(C1), 173.39(C36), 173.13(C39), 170.35(C52), 153.73(C50), 142.20(C27), 123.07(C28), 87.80(C46), 82.30(C44), 81.02(C2), 56.00(C48), 54.77(C43), 52.35(C9), 51.12(C25), 47.00(C18), 46.80(C30), 44.57(C14), 41.62(C12), 39.08(C23), 38.48(C5), 38.42(C6) 38.35(C21), 38.22(C47), 36.51(C31), 36.51(C19), 36.29(C11), 35.94(C53), 32.69(C54), 31.63(C20), 30.17(C37), 28.73(C38), 28.46(C16), 27.52(C17), 26.97(C4), 25.50(C29), 23.79(C15), 23.25(C8), 22.83(C7), 21.05(C22), 20.03(C10), 16.89(C24), 16.05(C13), 15.20(C32). LC/MS ESI$^+$: *m/z* calc.: 805.4626 found: 807.3162 $[M+H]^+$

Hybrid 25: Oleanolic acid (**9**) (500 mg, 1.095 mmol, 1. 0 equiv), succinic anhydride(109 mg, 1.095 mmol, 1. 0 equiv), pyridine(86 mg, 1.095 mmol, 1. 0 equiv), DMAP (133 mg, 1.095 mmol, 1. 0 equiv), DCM(10 ml). Oleanolic acid derivative (**24**) (300 mg, 0.54 mmol, 1.0 equiv), ursolic acid (246.62 mg, 0.54 mmol, 1.0 equiv), DCC (111.42 mg, 0.54 mmol, 1.0 equiv), DMAP (65.97 mg, 0.54 mmol, 1.0 equiv), DMF (10 ml). Column conditions: CH_2Cl_2/AcOEt 7:3. $Rf = 0.77$, m.p. 258–260 °C, white powder, Yield: 48 %. IR (ATR, γ cm^{-1}): 3451 OH cm^{-1}(carboxylic), 2937–2891 cm^{-1} (CH aliphatic), 1690 cm^{-1} (C=O), 1462 cm^{-1}(C=C alkene). ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 11.19(s, 1H, H-67), 11.18(s, 1H, H-34), 5.30–5.26(t, 1H, H-78), 5.26-5.22(t, 1H, H-77), 4.21–4.19(d, 1H, H-62), 4.18(d, 1H, H-2), 2.71–2.64(dd, 2H, H-38), 2.71-2.64(dd, 2H, H-37), 2.00 (dd, 1H, H-73), 2.90(dd, 1H, H-26), 1.75–1.73(d, 2H, H-55), 1.70-1.62(d, 2H, H-26). ^{13}CNMR (500 MHz, $CDCl_3$): δ (ppm) 180.52(C1), 176.91(C65), 173.39(C36), 173.39(C39), 144.76(C52), 143.16(C27), 125.42(C28), 122.63(C56), 81.98(C62), 81.98(C2), 60.81(C59), 55.24(C9), 47.63(C25), 47.63(C54), 46.56(C30), 46.56(C46), 41.64(C14), 41.01(C53), 41.01(C12), 39.30(C23), 39.30(C20), 38.75(C50), 38.42(C16), 37.07(C29), 33.04(C55), 32.44(C63), 30.65(C4), 28.96(C49), 28.80(C8), 28.09(C68), 27.17(C7), 25.91(C69), 23.57(C10), 22.94(C58), 22.94(C22), 18.30(C24), 18.30(C13), 16.99(C71), 15.31(C32), 14.13(C70). LC/MS ESI$^+$: *m/z* calc.: 994.7362 found: 994.7532 $[M+H]^+$

Hybrid 27: Oleanolic acid (500 mg, 1.09 mmol, 1.0 equiv), N^1,N^1-diethylpropane-1,3-diamine (111.37 mg, 1.09 mmol, 1.0 equiv), DCC (247.39 mg, 1.199 mmol, 1.1 equiv), HSU (125.45 mg, 1.09 mmol, 1.0 equiv), DMF (10 ml). ursolic acid (200 mg, 0.4 mmol, 1.0 equiv), oleanolic acid derivative (**20**) (227.4 mg, 0.4 mmol, 1.0 equiv), DCC (82.5 mg, 0.4 mmol, 1.0 equiv), DMAP (48.9 mg, 0.4 mmol, 1.0 equiv), DMF (10 ml). Column conditions: CH_2Cl_2/AcOEt 7:3. Rf = 0.58, m.p. 223–226 °C, white powder, Yield: 40 %. IR (ATR, γ cm^{-1}): 3531 (NH), 3363 cm^{-1} (OH), 2929–2901 cm^{-1} (CH aliphatic), 1708 cm^{-1} (C=O), 1455 cm^{-1}(C=C alkene). ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 7.80(s, 1H, H-66), 5.40-5.35(t, 1H, H-60), 5.30-5.26(t, 1H, H-28), 3.76-3.74(dd, 2H, H-68), 3.25-3.22(dd, 1H, H-37), 2.56-2.54(d, 1H, H-58). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 176.97(C1), 175.31(C36), 144.77(C59), 137.97(C27), 125.85(C28), 122.50(C60), 79.02(C37), 78.96(C2), 55.18(C9), 48.08(C25), 47.61(C18), 46.84(C30), 46.25(C62), 42.37(C52), 42.24(C57), 39.45(C48), 38.76(C14), 37.02(C12), 34.22(C46), 33.03(C40), 32.96(C68), 32.66(C21), 32.55(C5), 30.70(C63), 29.66(C31), 28.11(C11), 27.38(C45), 27.20(C76), 25.67(C20), 25.61(C16), 24.86(C50), 24.75(C4), 23.68(C17), 23.31(C51), 21.13(C44), 18.31(C10), 17.53(C24), 16.96(C64), 16.96(C32), 15.46(C74), 15.41(C75). LC/MS ESI$^+$: *m/z* calc.: 1006.8466 found: 1005.3568 $[M+H]^+$

Hybrid 36: Cholesterol (**8**) (1500 mg, 3.88 mmol, 1.0 equiv.), succinic anhydride (380 mg, 3.88 mmol, 1.0 equiv), pyridine (306 mg, 3.88 mmol, 1.0 equiv), DMAP (474.02 mg, 3.88 mmol, 1 equiv), DCM (5 ml).

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Cholesterol derivative (**22**) (400 mg, 0.82 mmol, 1 equiv), ursolic acid (375 mg, 0.83 mmol, 1.0 equiv), DMAP (100.18 mg, 0.82 mmol, 1 equiv), DCC (169.19 mg, 0.82 mml, 1 equiv) DMF (10 ml), Column conditions: Hexanes/AcOEt 1:1. Rf = 0.67, m.p. 115–117 °C, white powder, Yield: 50%. IR (ATR, γ cm^{-1}): 3547 NH, 3368 cm^{-1} (OH) carboxylic, 2928–2856 cm^{-1} (CH aliphatic), 1709 cm^{-1} (C=O), 1631 cm^{-1}(C=C alkene), (C=C) 1455 CM^{-1} aromatic. ^{1}H-NMR (500 MHz, CDCl3): δ (ppm) 10.62(s, 1H, H-34), 5.24(t, 1H, H-28), 4.13–4.12(d, 1H, H-43), 4.10-4.09 (d, 1H, H-2), 3.41-3.40(d, 2H, H-38), 3.39-3.38(d, 2H, H-38), 3.18-3.15(dd, 1H, H-26), 2.27-2.21(dd, 2H, H-29), 1.59-156 (m, 2H, H-4), 1.54-1.41(m, 2H, H-48), 1.40–1.31(m, 2H, H-44), 1.24-1.23(d, 1H, H-65), 1.21-1.04 (t, 2H, H-19). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 177.45(C1), 172.70(C36), 171.73(C39), 141.48(C27), 125.38(C28), 77.62(C2), 70.73(C43), 59.96(C9), 56.79(C25), 56.18(C18), 55.32(C30), 52.99(C58), 50.35(C56), 47.59(C14), 47.36(C45), 42.42(C49), 42.22(C55), 41.99(C50), 39.81(C12), 39.36(C23), 39.01(C64), 38.96(C5), 38.62(C21), 37.36(C46), 36.71(C60), 36.43(C19), 36.08(C31), 35.71(C61), 31.92(C57), 31.75(C11), 29.47(C44) 29.32(C59), 28.23(C20), 28.20(C52), 28.02(C37), 27.93(C38), 27.93(C48), 27.84(C65), 27.79(C16), 27.21(C53), 24.11(C51), 24.05(C53), 23.11(C17), 23.05(C29), 22.17(C4), 22.17(C62), 21.93(C7), 20.92(C8), 20.57(C67), 18.90(C66), 18.25(C10), 16.81(C22), 16.64(C24), 15.03(C13), 13.58(C63), 11.34(C32). LC/MS ESI^{+}: *m/z* calc.: 898.7207 found: 899.6099 $[M+H]^{+}$

Hybrid 34: 4.7 Dichloroquinoline (500 mg, 2.52 mmol, 1.0 equiv), 2-(2-aminoethoxy)ethanol (265.44 mg, 2.52 mmol, 1.0 equiv). Ursolic acid (400 mg, 0.88 mmol, 1.0 equiv), 2-(2-(7-chloroquinolin-4-ylamino)ethoxy)ethanol (**35**) (195.02 mg, 0.8 mmol equiv), DMF (5 mL), DMAP (97.74 mg, 0.8 mmol, equiv), DCC (181.56 mg, 0.88 mmol, 1.0 equiv). Column conditions: Hexanes/AcOEt 6:4. R_f = 0.57, m.p. 190–195 °C, white powder, Yield: 62%. IR (ATR, γ cm^{-1}): 3547 NH, 3382 cm^{-1} (OH) carboxylic, 2930–2903 cm^{-1} (CH aliphatic), 1666 cm^{-1} (C=O), 1491 cm^{-1}(C=C alkene), 1456 cm^{-1}(C=C) aromatic. ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 8.82-8.81(d, 1H, H-47), 8.22-8.20(d, 1H, H-48), 8.15(s, 1H, H-45), 7.64(d, 1H, H-40), 7.62(dd, 1H, H-39), 7.52-7.51(d, 1H, H-44),5.28-5.25(t, 1H, H-50), 4.51-4.47(t, 2H, H-36), 3.65(t, 2H, H-37), 3.52-3.49(dd, 1H, H-2), 2.91-2.87(dd, 1H, H-26). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 178.66(C1), 150.97(C43), 149.47(C27), 142.70(C41), 136.55(C39), 128.75(C45), 125.60(C48), 125.04(C47), 121.41(C40), 105.57(C44), 78.57(C2), 63.66(C36), 54.90(C9), 53.82(C25), 49.11(C18), 48.21(C30), 42.08(C37), 42.01(C14), 40.07(C12), 39.60(C23), 39.59(C6), 38.39(C21), 37.98(C5), 36.50(C31), 36.42(C19), 33.50(C11), 30.21(C20), 28.01(C16), 27.72(C4), 25.88(C17), 24.34(C15), 24.34(C29), 23.78(C7), 23.78(C8), 18.88(C10), 18.79(C22), 17.94(C24), 17.50(C13), 16.09(C32). LC/MS ESI^{+}: *m/z* calc.: 659.4105 found: 660.4881 $[M+H]^{+}$

Hybrid 36: 4.7 dichloroquinoline (**10**) (500 mg, 2.52 mmol, 1.0 equiv), 2-aminoethanol (153.93 mg, 2.52 mmol, 1.0 equiv). Ursolic acid (400 mg, 0.88 mmol, 1.0 equiv), 2-(7-chloroquinolin-4-ylamino) ethanol (**33**) (195.03 mg, 0.88 mmol, 1.0 equiv), DCC (199.73 mg, 1.97 mmol, 1.1 equiv), DMAP (229.68 mg, 1.88 mmol, 1.0 equiv). Column conditions: Hexanes/AcOEt 7:3. R_f = 0.9, m.p. 170–173 °C, white powder, Yield: 54%. IR (ATR, γ cm^{-1}): 3547 NH, 3365 cm^{-1} (OH) carboxylic, 2928–2856 cm^{-1} (CH aliphatic), 1709 cm^{-1} (C=O), 1631 cm^{-1}(C=C alkene), (C=C) 1455 CM^{-1} aromatic. ^{1}H-NMR (500 MHz, $DMSO_{-6}$): δ (ppm), 8.82-8.81(d, 1H, H-43), 8.22–8.22(d, 1H, H-51), 8.16–8.15(d, 1H, H-50), 7.64–7.62(dd, 1H, H-42), 7.51(s, 1H, H-48), 6.12(s, 1H, H-41), 5.27(t, 1H, H-28), 4.51–4.47(t, 2H, H-36), 3.79-3.77(t, 2H, H-37), 3.67–3.64(t, 2H, H-39), 3.52–3.50(t, 2H, H-40), 2.89-2.87(d, 1H, H-26), 2.19(s, 1H, H-3), 1.92–1.71(d, 2H, H-29). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 182.14(C1), 150.99(C43), 149.47(C47), 142.71(C45), 138.93(C27), 136.54(C49), 128.75(C48), 128.67(C51), 125.61(C28), 125.04(C50), 124.85(C46), 121.42(C42), 78.98(C2), 72.71(C39), 72.38(C37), 70.25(C36), 61.75(C9), 55.24(C25), 53.18(C18), 48.04(C30), 47.61(C40), 42.20(C14), 41.42(C12), 39.52(C23), 39.29(C6), 39.03(C21), 38.75(C5), 38.63(C31), 37.01(C19), 33.13(C11), 28.16(C20), 27.25(C16), 23.51(C4), 23.51(C15), 21.29(C29), 18.38(C8), 17.29(C7), 17.12(C10), 15.65(C22), 15.47(C32). LC/MS ESI^{+}: *m/z* calc.: 704.4320 found: 704.5134 $[M+H]^{+}$

Hybrid 29: Ciprofloxacin (**11**) (500 mg, 1.51 mmol, 1 equiv), 2-chloroacetyl chloride(170.54 mg, 1.51 mmol, 1 equiv), triethylamine (0.64 ml, 5.66 mmol, 1.0 equiv), tetrahydrofuran (THF)(4 ml). Ciprofloxacin derivative (**28**) (300 mg, 0.74 mmol, 1.0 equiv), ursolic acid (335.96 mg, 0.74 mmol, 1.0 equiv), DCC (167.13 mg, 0.81 mmol, 1.0 equiv), DMAP (90.41 mg, 0.74 mmol, 1.0 equiv) and DMF (10 ml). Column conditions: Hexanes/AcOEt 7:3. Rf = 0.44, m.p. 217–220 °C, white powder, Yield: 58%. IR (ATR, γcm^{-1}): 3410 OH cm^{-1}(carboxylic), 2929–2894 cm^{-1} (CH aliphatic), 1691 cm^{-1} (C=O), 1462 cm^{-1}(C=C alkene), (C=C) 1378 CM^{-1} aromatic. ^{1}H-NMR (500 MHz, DMSO-

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6): δ (ppm) 11.02(s, 1H, H-46), 10.70(s, 1H, H-31), 8.70(s, 1H, H-64), 7.97–7.95(s, 1H, H-39), 7.62–7.61(s, 1H, H-36), 5.14(t, 1H, H-63), 3.52–3.47(d, 1H, H-2), 3.34–3.27(dd, 4H, H-54/50), 3.01(s, 2H, H-58), 2.13-2.11(d, 4H, H-51/53). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 184.36(C38), 178.67(C1), 172.13(C35), 166.27(C58), 148.58(C46), 139.60(C40), 138.67(C26), 131.80(C44), 125.06(C27), 107.39(C39), 107.22(C45), 98.77(C47), 77.33(C2), 55.28(C57), 52.88(C24), 40.57(C50), 39.57(C52), 38.85(C49), 37.02(C53), 28.74(C13), 23.74(C11), 21.52(C22), 17.46(C4), 17.40(C20), 16.69(C30). LC/MS ESI$^+$: *m/z* calc.: 809.4885 found: 810.5376 $[M+H]^+$

Hybrid 31: Hybrid **13** (300 mg, 0.51 mmol, 1.0 equiv), ferrocene-4 ketobutanoic acid (**6**) (145.91 mg, 0.51 mmol, 1.0 equiv), DCC (115.75 mg, 1.56 mmol, 1.1 equiv), DMAP (62.31 mg, 0.51 mmol, 1.0 equiv), DMF (10 ml). Column conditions: Hexanes/AcOEt 7:3. R_f = 0.56, m.p. 100–105 °C, brown powder, Yield: 52%. IR (ATR, γ cm^{-1}): 2925–2857 cm^{-1} (CH aliphatic), 1744 cm^{-1} (C=O), 1466 cm^{-1}(C=C alkene), 1382 cm^{-1} (C=C) aromatic. ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 7.06(d, 1H, H-57), 6.75(d, 1H, H-56), 6.69(s, 1H, H-54), 5.27(t, 1H, H-28), 4.85(s, 2H, H-42/46), 4.55(s, 2H, H-43/44), 4.27(s, 3H, H-47-51), 2.24(d, 1H, H-3), 3.11(m, 1H, H-6), 2.85(t, 2H, H-39), 2.78(t, 2H, H-37), 2.37(s, 3H, H-59).2.24(dd, 1H, H-26), 2.07-2.04(t, 2H, H-29). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 202.25(C39), 175.75(C2), 173.39(C35), 153.67(C53), 148.43(C55), 137.91(C27), 130.78(C57), 130.78(C58), 125.89(C28), 120.80(C56), 118.73(C54), 79.11(C42), 78.22(C50), 72.36(C47), 69.97(C45), 69.26(C46), 58.490(C48), 55.26(C49), 52.66(C3), 47.97(C9), 47.59(C25), 38.84(C18), 34.08(C30), 33.67(C14), 28.14(C12), 27.69(C23), 23.97(C5), 23.59(C21), 21.15(C31), 18.40(C60), 16.99(C11), 16.96(C37), 15.60(C20), 15.48(C16), 15.26(C17). LC/MS ESI$^+$: *m/z* calc.: 856.5668 found: 855.3790 $[M-H]^+$

Hybrid 32: Hybrid **13** (300 mg, 0.51 mmol, 1.0 equiv), Cholesterol derivative (**22**) (76.50 mg, 0.51 mmol, 1.0 equiv), DCC (115.75 mg, 1.56 mmol, 1.1 equiv), DMAP (62.31 mg, 0.51 mmol, 1.0 equiv), DMF (10 ml). Column conditions: Hexanes/AcOEt 7:3. R_f = 0.81, m.p. 130–135 °C, white powder, Yield: 55%. IR (ATR, γ cm^{-1}): 2925–2956 cm^{-1} (CH aliphatic), 1738 cm^{-1} (C=O), 1464 cm^{-1}(C=C alkene), 1465 cm^{-1} (C=C) aromatic ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 6.99-6.98(d, 1H, H-71), 6.71(s, 1H, H-69), 6.64–6.62(d, 1H, H-72), 5.34–5.33(d, 1H, H-78), 4.13-4.12(d, 1H, H-44), 4.10–4.09(d, 1H, H-2), 3.65(s, 3H, H-72), 3.42–3.41(d, 2H, H-39), 3.41 −3.40(d, 2H, H-38), 3.18-3.16(dd, 1H, H-26), 2.86(m, 1H, H-75), 2.18–2.76(d, 6H, H-77/76). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 177.18(C1), 172.70(C37), 171.46(C40), 152.72(C36), 148.09(C70), 141.48(C27), (C72), 129.09(C72), 125.38(C73), 123.89(C71), 123.03(C38), 120.66(C68), 77.62(C2), 70.73(44), 59.86(C9), 56.79(C25), 56.18(C30), 55.32(C18), 52.99(C59), 50.35(C57), 47.59(C14), 47.36(C46), 42.42(C50), 41.99(C51), 39.81(C12), 39.36(C47), 39.01(C56), 38.96(C23), 38.62(C65), 37.37(C5), 36.71(C6), 36.43(C61), 36.08(C21), 35.71(C31), 31.92(C62), 31.75(C58), 28.23(C75), 27.79(C19), 24.11(C11), 24.05(C45), 23.63(C60), 23.11(C55), 23.05(C20), 22.17(C38), 21.93(C39), 20.92(C53), 20.57(C16). LC/MS ESI$^+$: *m/z* calc.: 1030.7989 found: 1030.6869 $[M-H]^+$

Hybrid 30: Hybrid **13** (300 mg, 0.51 mmol, 1.0 equiv), cinnamic acid (**4**) (76.50 mg, 0.51 mmol, 1.0 equiv), DCC (115.75 mg, 1.56 mmol, 1.1 equiv), DMAP (62.31 mg, 0.51 mmol, 1.0 equiv), DMF (10 ml). (ATR, γ cm^{-1}): Column conditions: Hexanes/AcOEt 7:3. R_f = 0.66, m.p. 114–115 °C, white powder Yield: 66%. IR (ATR, γ cm^{-1}): 2930–2857 cm^{-1} (CH aliphatic), 1683 cm^{-1} (C=O), 1607 cm^{-1}(C=C alkene), 1452 cm^{-1} (C=C) aromatic. ^{1}H-NMR (500 MHz, DMSO-6): δ (ppm) 7.94–7.81(d, 1H, H-51), 7.60(dd, 2H, H-52/54), 7.45–7.44(dd, 1H, H-53), 7.44(d, 1H, H-55), 7.07–7.06(d, 1H, H-41), 6.76–6.74(d, 1H, H-42), 6.69(s, 1H, H-39), 6.50–6.67(d, 1H, H-45), 5.28(t, 1H, H-56), 3.28-3.24(dd, 1H, H-25), 2.88–2.82(m, 1H, H-45), 2.41-2.37(d, 2H, H-48), 2.41–2.37(d, 2H, H-49), 2.24(s, 3H, H-44). ^{13}CNMR (500 MHz, CDCl3): δ (ppm) 171.75(C1), 170.08(C36), 153.63(C38), 148.43(C40), 147.03(C26), 134.07(C50), 130.79(C42), 130.72(C43), 128.96(C52), 128.35(C27), 125.89(C51), 120.78(C54), 118.75(C55), 117.22(C53), 112.99(C27), 117.22 (C41), 112.99 (C39), 79.15(C2), 55.24(C8), 52.62(C24), 48.02(C17), 47.58(C29), 39.54(C13), 39.05(C11), 38.84(C22), 38.75(C4), 38.64(C5), 36.99(C20), 36.70(C30), 33.67(C18), 32.96(C45), 31.92(C48), 30.61(C10), 29.69(C18), 28.13(C45), 28.00(C48), 27.33(C10), 27.18(C19), 24.72(C49), 23.98(C15), 23.63(C16), 23.31(C14), 21.16(C28), 18.32(C3), 16.98(C47), 15.60(C46), 15.47(C7), 15.27(C6). LC/MS ESI$^+$: *m/z* calc.: 720.5118 found: 721.2905 $[M+H]^+$

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Biological Activities

Antibacterial Activities

The minimum inhibitory concentration (MIC) of the studied hybrid molecules was carried out following Fonkui *et al.*, (2018).[66] Stock solutions were prepared by adding 3.4 mL of DMSO to each tube containing 4 mg of the synthesized compounds. These solutions were then serially diluted (6 times) in 100 µL of nutrient broth in 96 well plates to the desired concentrations (500, 250, 125, 62.5, 31.25 and 15.625 µg/mL). Then after, 100 µL of each of these solutions was placed in duplicate and seeded with 100 µL of an overnight bacterial culture brought to 0.5 Mc Farland in nutrient broth. Streptomycin (STM), ampicillin (AMP) and nalidixic acid (NLD) were used as positive control and negative control was prepared to contain 50% nutrient broth in DMSO.

The antibacterial activities of the synthesized hybrids against five Gram-positive *Bacillus subtilis* (**ATCC19659**) (BS), *Enterococcus faecalis* (**ATCC13047**) (EF), *Staphylococcus epidermidis* (**ATCC14990**) (SE), *Staphylococcus aureous* (**ATCC25923**) (SA), *Mycobaterium smegmatis* (**MC2155**) (MS) and six Gram-negative bacteria *Enterobacter cloacae* (**ATCC13047**) (ECL), *Proteus vulgaris* (**ATCC6380**) (PV), *Klebsiella oxytoca* (**ATCC8724**) (KO), *Pseudomonas aeruginosa* (**ATCC27853**) (PA), *Proteus mirabilis* (**ATCC7002**) (PM), *Escherichia coli* (**ATCC25922**) (EC) were evaluated via Disc diffusion method. *Table 1* shows the Minimal Inhibitory Concentrations (MIC) of these hybrid compounds.

In vitro *Cytotoxicity Evaluation*

The MCF-7, MD-MBA-231 and HeLa cells obtained from Cellonex, RSA, were cultured in Dulbecco's Modified Eagle Medium (Hyclone, United States) supplemented with 10% heat-inactivated Foetal Bovine Serum (Hyclone, United States) and 1% Penicillin-Streptomycin-Neomycin (Gibco, Auckland, New Zealand). The cells were kept in a 5% CO_2 incubator at 37 °C. Raw 264.7 cells were used as non-cancerous controls. The cytotoxicity of the synthesized ester-linked ursolic acid-based hybrid compounds was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In these cytotoxicity assays, cells were seeded overnight at a concentration of 1×10^4 cells/well in a 96-well culture plate and then treated with the different compounds. Following treatment, 30 µL of MTT (5 mg/mL in PBS) MTT reagent (Thermo Fischer Scientific, United States) was added to each well to a final volume of 300 µL and then incubated in the CO_2 incubator for an additional 3 h. Following incubation, the culture media was discarded and replaced with 100 µl DMSO (dimethyl sulfoxide, 99.99%) was added and the plate was placed in the dark for 1 h at 25 °C. The color intensity of the purple formazan formed was read at 570 nm using a microplate reader (GloMax®, Promega). All experiments were conducted in triplicates and repeated three times. The percentage of viable cells was calculated using the following formula:

$$\%\ Cell\ viability = \frac{Absorbance_{560\ nm} Treated\ cells}{Absorbance_{560\ nm} Untreated\ cells} \times 100$$

Molecular Docking Studies

To determine the protein-ligand interaction, a molecular docking study was performed on the newly designed compounds using Glide modules of the Schrödinger Maestro software package (Schrödinger Release 2021-4: Maestro, Schrödinger, LLC, New York, NY, 2020. version 13.0). The hybrid compounds and the parent ursolic acid were docked into the epidermal growth factor receptor (EGFR) kinase in complex with a 4-anilinoquinazoline inhibitor (PDB ID: 1 M17). EGFR is frequently overexpressed in human tumors, and based on its role in promoting cell proliferation, it has been studied as an excellent target for cancer therapy.[67] Therefore, the crystal structure of 1 M17 was obtained from the RCSB Protein Data Bank (http://www.RCSB.org). The protein structure was further prepared, optimized, and minimized using the 'protein preparation wizard' by retaining the default settings for rectifying the PDB structure for the docking process.[68] On the other hand, the two-dimensional structure of the ligands was generated using Chem Draw Ultra 0.7 and subsequently converted to *SDF format* using the Open Babel 2.4.1 program.[69] The ligands were optimized using the LigPrep module which generates low-energy 3D structure using the OPLS4 force field. Thereafter, the receptor grid generation panel was used to define a grid box having a size of 20 Å around the co-crystallized ligand, and with the center coordinates (x = 21.29, y = 0.54, z = 52.35). Finally, molecular docking simulation was carried out on the ligand docking panel by selecting the Extra Precision (XP) mode.[68,70]

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Author Contribution Statement

All the authors contributed equally to the manuscript.

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