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Cholic Acid Conjugates in Modern Therapeutics: Advances in Anticancer, Antimicrobial, and Targeted Drug Delivery Applications

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Summary: Cholic acid, a primary bile acid, has emerged as a versatile scaffold for novel therapeutic design due to its unique amphiphilic structure, which imparts both hydrophilic and hydrophobic characteristics. These properties not only enable cholic acid to play essential roles in lipid absorption and cellular signaling via various receptors but also allow extensive chemical modifications that can be harnessed for drug development. Recent advances in medicinal chemistry have demonstrated that cholic acid derivatives and their conjugates can be engineered to enhance drug delivery, improve anticancer efficacy, and overcome antimicrobial resistance. The hydrophobic steroid nucleus of cholic acid, combined with its hydrophilic hydroxyl and carboxyl groups, plays essential role in the production of a broad array of conjugates and hybrid molecules. For example, cholic acid has been conjugated with cytotoxic agents such as cytarabine and tamoxifen, resulting in prodrugs with superior liver-targeting capabilities and enhanced anticancer activity against diverse cell lines, including HL-60, HCT116, MCF-7, and MDA-MB-231. In addition, linkage with platinum drugs, organotin compounds, and artemisinin analogues has yielded compounds that not only exhibit potent cytotoxicity but also overcome multidrug resistance in cancer cells. Beyond oncology, the inherent properties of cholic acid have been exploited to develop effective antimicrobial therapies. Cholic acid-based hybrids have demonstrated significant activity against drug-resistant pathogens, while innovative delivery systems—such as thiomeric micelles encapsulating metallic nanoparticles—have further advanced targeted drug delivery and controlled release. Collectively, these studies underscore the promise of cholic acid as a multifaceted platform in drug design. By fine-tuning chemical linkers and conjugation strategies, researchers have developed a new generation of prodrugs and hybrid molecules that improve therapeutic efficacy while minimizing adverse effects. This review synthesizes current findings on the structure–activity relationships of cholic acid derivatives, addresses challenges in their synthesis and clinical translation, and outlines promising future directions in the field of cholic acid-based therapeutics.

Keywords: Bile Acid Derivatives, Cholic Acid Conjugates, Anticancer Agents, Antimicrobial Activity, Targeted Drug Delivery

Introduction

Bile acids are a broad class of amphiphilic compounds that are vital for lipid digestion, metabolic regulation, and cellular signaling. Their unique structure—featuring a hydrophobic steroid nucleus combined with hydrophilic hydroxyl and carboxyl groups—has spurred significant interest in their application as scaffolds for drug development. Among the bile acids, cholic acid conjugates have emerged as particularly intriguing components in the design of targeted therapeutics for cancer and infectious diseases. The multifaceted biological functions of bile acids extend beyond their classical roles in digestion. Recent advances in medicinal chemistry have harnessed these molecules to enhance drug delivery, improve pharmacokinetic profiles, and mitigate systemic toxicity. The primary focus of the current review is on the synthesis, functionalization, as well as biological investigation of bile acid derivatives, with a special emphasis on cholic acid conjugates, which demonstrate superior targeting capabilities and therapeutic efficacy in various preclinical models.

Cholic acid as a key component of the human digestive system acts as a versatile framework for the development of new therapeutic substances. Its distinct amphiphilic structure—comprising a hydrophobic steroid nucleus and hydrophilic functional groups—facilitates both biological activity and chemical modification. Recent research has focused on leveraging cholic acid's unique properties

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to create conjugates with improved drug delivery, enhanced anticancer activity, and potent antimicrobial effects. Cholic acid derivatives have gained a lot of interest because of their ability to modulate cellular pathways and target specific tissues, such as the liver and colon. By conjugating cholic acid with various bioactive moieties, researchers have developed a range of prodrugs and hybrid compounds that offer significant advantages over conventional therapies. This review highlights the potential of cholic acid-based therapeutics to revolutionize treatment strategies for cancer and infectious diseases by summarizing current developments in their synthesis and biological evaluation. Cholic acid and chenodeoxycholic acid are both derived from cholesterol. In humans, these two acids are present in equal amounts and are converted into amino-conjugated bile acids [1]. Cholic acid, also known as 4-(3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl) pentanoic acid, is an essential bile acid [2].

Many animals, including humans, naturally produce this type of endogenous steroid [3]. The biological activity of a steroid molecule is significantly altered when one or more of its carbon atoms are replaced by heteroatoms [4-6]. In supramolecular chemistry, cholic acid is increasingly being investigated as a structural component [7]. Cholic acid contains two axial hydroxyl groups (at C_7 and C_2) and one equatorial hydroxyl group (at C_3). In cyclohexane derivatives, equatorial hydroxyl groups esterify more readily than axial hydroxyl groups. A similar process has been observed in cholic acid, where the equatorial hydroxyl group is selectively acylated. Early bile acid research indicates that the axial hydroxyl group at C_7 is more reactive than the one at C_2, suggesting that steric effects alone cannot fully explain this increased activity. These aspects of cholic acid's reactivity have been confirmed through various synthetic investigations [8]. Cholic acid is primarily produced in the liver [9]. It also serves as a surfactant, aiding in the solubilization of fats and vitamins to facilitate their absorption in the colon [10]. This function is maintained through the formation of water-soluble salts by conjugation with taurine or glycine, which enhances fat absorption and cholesterol excretion. These conjugated bile acids then form micelles around lipophilic molecules, promoting emulsification and absorption [11, 12].

Bile acid derivatives hold great promise as drug carriers due to their amphiphilic nature [13]. The widespread use of antibacterial agents has led to increased antibiotic resistance [14]. To address this challenge, several cholic acid derivatives have been synthesized to enhance bacterial cell wall permeability and combat antibiotic resistance [15]. Bile acids (BAs) serve as excellent building blocks for antimicrobial development due to their large hydrophobic steroid backbone and facial amphiphilic structure, which is similar to that of antimicrobial peptides (AMPs) [16, 17]. This structural similarity facilitates their penetration into bacterial membranes. For example, squalamine, a steroidal compound derived from the dogfish shark, has demonstrated broad-spectrum antibacterial activity due to its comparable steroid structure [18].

Bile acids also exhibit various biological activities, including anticancer properties [19], and can be used to reduce the cytotoxicity of anticancer drugs [20]. Numerous studies demonstrate that bile acid–based compounds exert antitumor effects through diverse but complementary mechanisms. BA derivatives suppress proliferation of biliary duct carcinoma cells by inhibiting the EGF–EGFR axis, thereby enhancing E-cadherin, reducing N-cadherin, and blocking epithelial–mesenchymal transition and invasiveness, with additive efficacy when combined with conventional EGFR inhibitors [21]. These derivatives act through a distinct mitochondrial dependent mechanism, where their amphiphilic structure promotes mitochondrial accumulation, resulting in membrane potential collapse, oxidative stress, cytochrome C release, and caspase mediated apoptosis [22]. Similarly, folate-conjugated cholic-acid–polyethylenimine micelles provide targeted and synergistic antitumor action by facilitating receptor-mediated uptake and co-delivery of doxorubicin and oncogene-silencing siRNA, producing DNA damage and transcriptional suppression [23]. They disrupt microtubule or DNA-associated processes, induce oxidative stress, and activate intrinsic apoptotic pathways against various cancer cell lines like PC3, Bcap37, and BgC823 cells [24]. Collectively, these bile acid–derived and structurally related compounds employ distinct yet converging mechanisms, ranging from receptor signaling inhibition to mitochondrial disruption and gene silencing to effectively suppress tumor growth.

By focusing on cholic acid as a versatile scaffold for drug conjugation, the review aims to elucidate the structure–activity relationships that underpin enhanced anticancer and antimicrobial activities, as well as improved targeted drug delivery. The article discusses diverse synthetic strategies employed to generate cholic acid conjugates with cytotoxic agents, platinum drugs, organotin compounds, and other bioactive molecules, highlighting how these modifications improve

pharmacokinetic profiles, reduce systemic toxicity, and increase specificity toward malignant cells and drug-resistant pathogens. Ultimately, this review seeks to guide future research directions by addressing the challenges in clinical translation and by proposing novel approaches for developing next-generation prodrugs and hybrid therapeutics for cancer and infectious diseases.

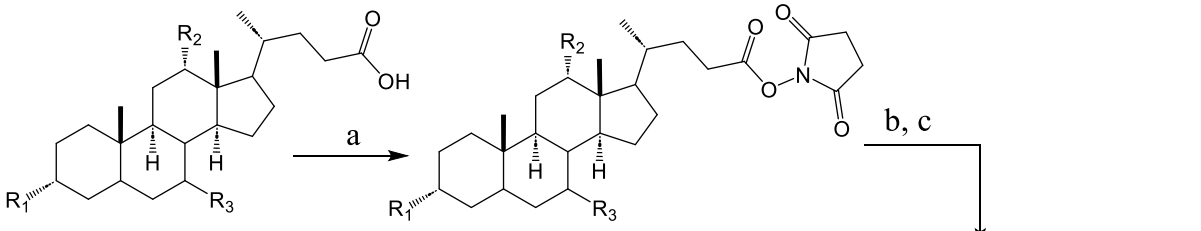
Antimicrobial activities

Cholic acid-based mimics of squalamine and polymyxin B

In an effort to discover novel antibacterial drugs, Savage et al. synthesized cholic acid-based mimics of squalamine and polymyxin B. While the polymyxin and sulfate functional groups present in the original antibiotics were retained in the squalamine mimics, their positions relative to the steroid nucleus were modified. The cholic acid-based squalamine mimics were synthesized in two or three steps (Scheme 1). First, the C_{24} site of cholic acid was modified to a succinimidyl ester. The intermediate alcohol-containing molecule was then treated with a sulfur trioxide-pyridine complex to generate sulfonated derivatives. Finally, an amide bond was formed by reacting the intermediate with the appropriate amine, yielding the squalamine mimics (Fig 1).

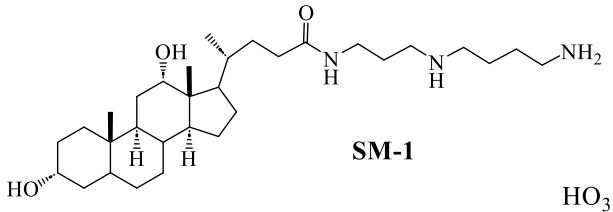
The squalamine mimic **SM-5** exhibited MIC values of approximately 100 µg/mL against all tested Gram-negative strains while also showing moderate activity against several Gram-positive bacteria. **SM-2** displayed similar characteristics but was generally less active than **SM-5**. Conversely, squalamine mimics lacking a sulfate group, such as **SM-1**, **SM-6**, and **SM-3**, exhibited activity against both Gram-positive and Gram-negative organisms. However, despite the absence of a sulfate group, **SM-3** was relatively inactive against Gram-negative bacteria.

Polymyxin mimics were synthesized from a common intermediate. Among these, **PM-8**, **9**, **10**, **11**, and **12** were particularly significant due to their enhanced antimicrobial activity (Fig 2). The PMB mimics consisted of a hydrophobic chain, primary amine groups, and the preservation of key functional groups found in polymyxin-family antibiotics. PMB mimics, specifically **PM-10** to **PM-12**, contain longer hydrophobic chains, enhancing their activity against Gram-negative bacteria. Notably, the hydrophobic chains in **PM-8** and **PM-9** significantly increase their efficacy against certain Gram-negative pathogens [25]. Many mimic compounds exhibit strong antibacterial activity and selectivity for bacterial cells, primarily by disrupting or permeabilizing bacterial membranes [26].



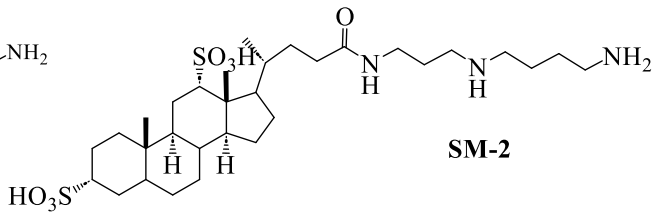
	R_1	R_2	R_3
SM-1	OH	OH	H
SM-2	SO_3H	SO_3H	H
SM-3	OH	OH	OH
SM-4	SO_3H	SO_3H	SO_3H
SM-5	SO_3H	H	H
SM-6	OH	H	H
SM-7	OH	H	OH

Scheme-1: Preparation of squalamine mimics. Reagents: (a) dicyclohexylcarbodiimide, N-hydroxysuccinimide, Tetrahydrofuran (b) SO_3-pyridine, Dimethyl formamide (c) spermine, $CHCl_3$.



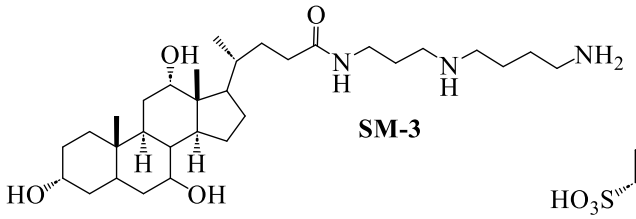
SM-1

(4*R*)-*N*-(3-((4-aminobutyl)amino)propyl)-4-((3*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide



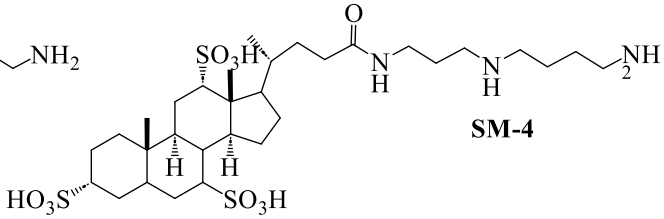
SM-2

(3*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-17-((*R*)-5-((3-((4-aminobutyl)amino)propyl)amino)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,12-disulfonic acid



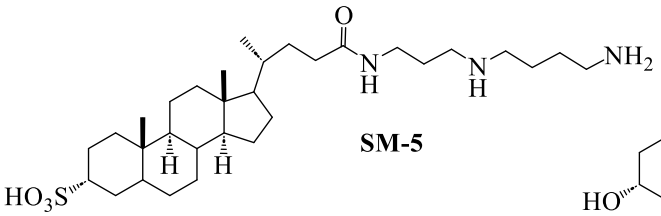
SM-3

(4*R*)-*N*-(3-((4-aminobutyl)amino)propyl)-4-((3*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide



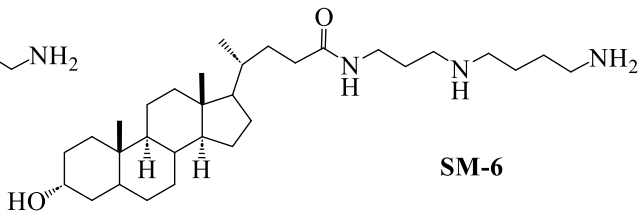
SM-4

(3*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-17-((*R*)-5-((3-((4-aminobutyl)amino)propyl)amino)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7,12-trisulfonic acid



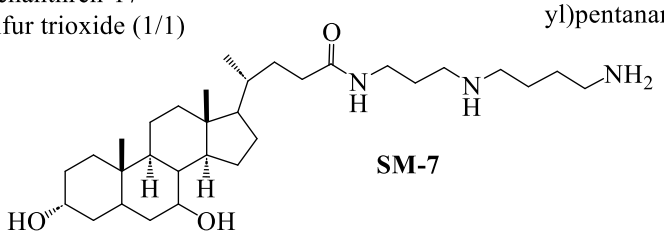
SM-5

(4*R*)-*N*-(3-((4-aminobutyl)amino)propyl)-4-((9*S*,10*S*,13*R*,14*S*)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide--sulfur trioxide (1/1)



SM-6

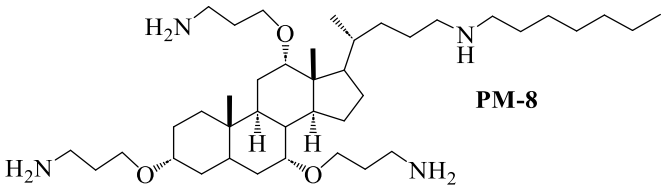
(4*R*)-*N*-(3-((4-aminobutyl)amino)propyl)-4-((3*R*,9*S*,10*S*,13*R*,14*S*)-3-hydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide



SM-7

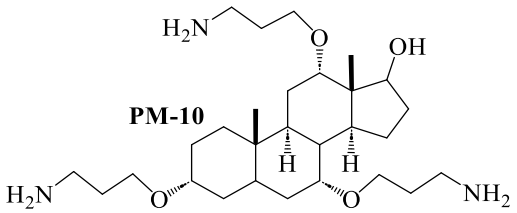
(4*R*)-*N*-(3-((4-aminobutyl)amino)propyl)-4-((3*R*,9*S*,10*S*,13*R*,14*S*)-3,7-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanamide

Fig. 1: Structures of squalamine mimics alongwith IUPAC names.



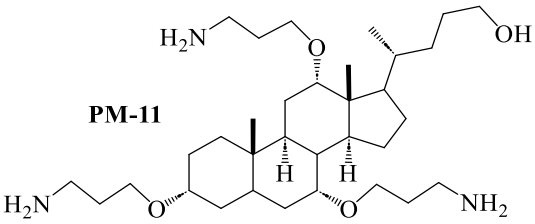
PM-8

3,3',3''-(((3*R*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-17-((*R*)-5-(heptylamino)pentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7,12-triyl)tris(oxy))tris(propan-1-amine)



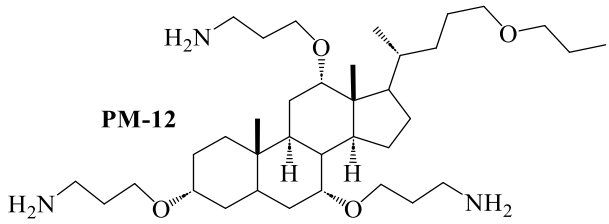
PM-10

(3*R*,7*R*,9*S*,10*S*,12*S*,13*S*,14*S*)-3,7,12-tris(3-aminopropoxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-ol



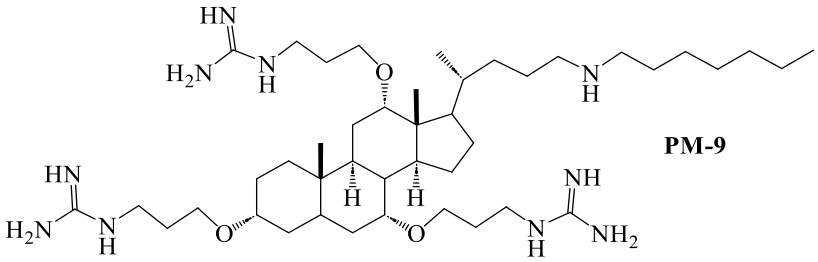
PM-11

(4*R*)-4-((3*R*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-3,7,12-tris(3-aminopropoxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentan-1-ol



PM-12

3,3',3''-(((3*R*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-10,13-dimethyl-17-((*R*)-5-propoxypentan-2-yl)hexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,7,12-triyl)tris(oxy))tris(propan-1-amine)



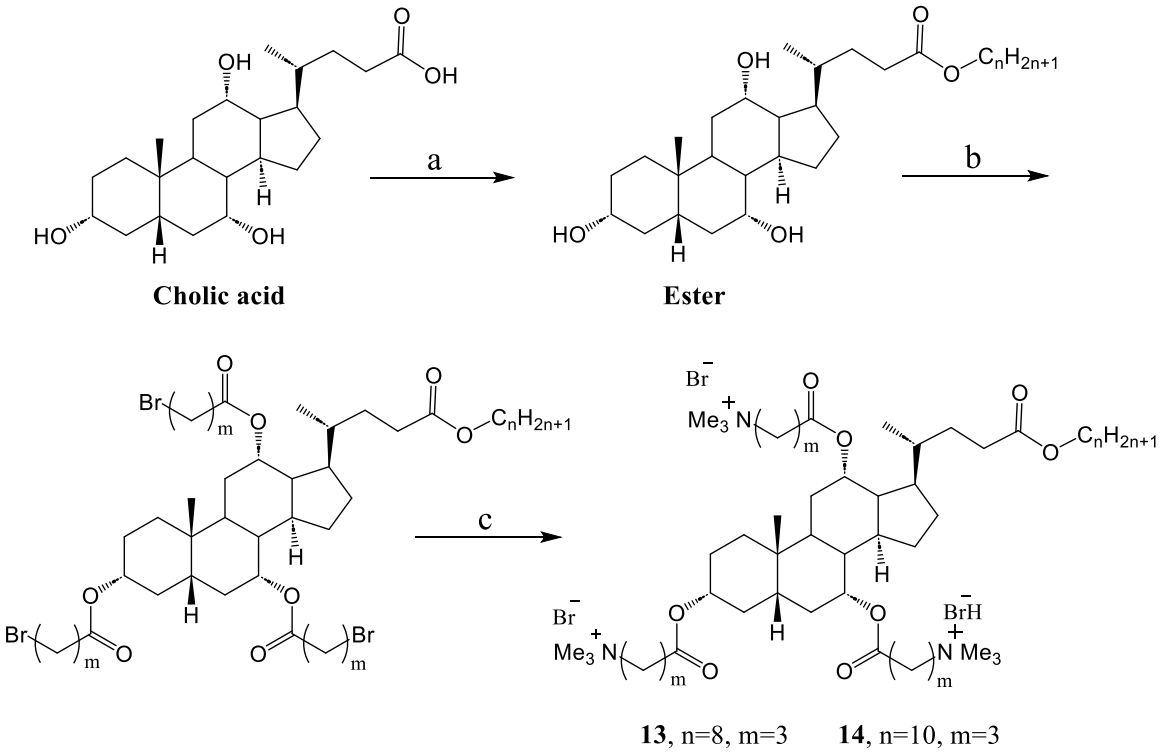
PM-9

1,1'-((((3*R*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-7-(3-guanidinopropoxy)-17-((*R*)-5-(heptylamino)pentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,12-diyl)bis(oxy))bis(propane-3,1-diyl))diguanidine

Fig. 2: Structures of Polymyxin B mimics alongwith IUPAC names.

Cholic acid derived amphiphiles

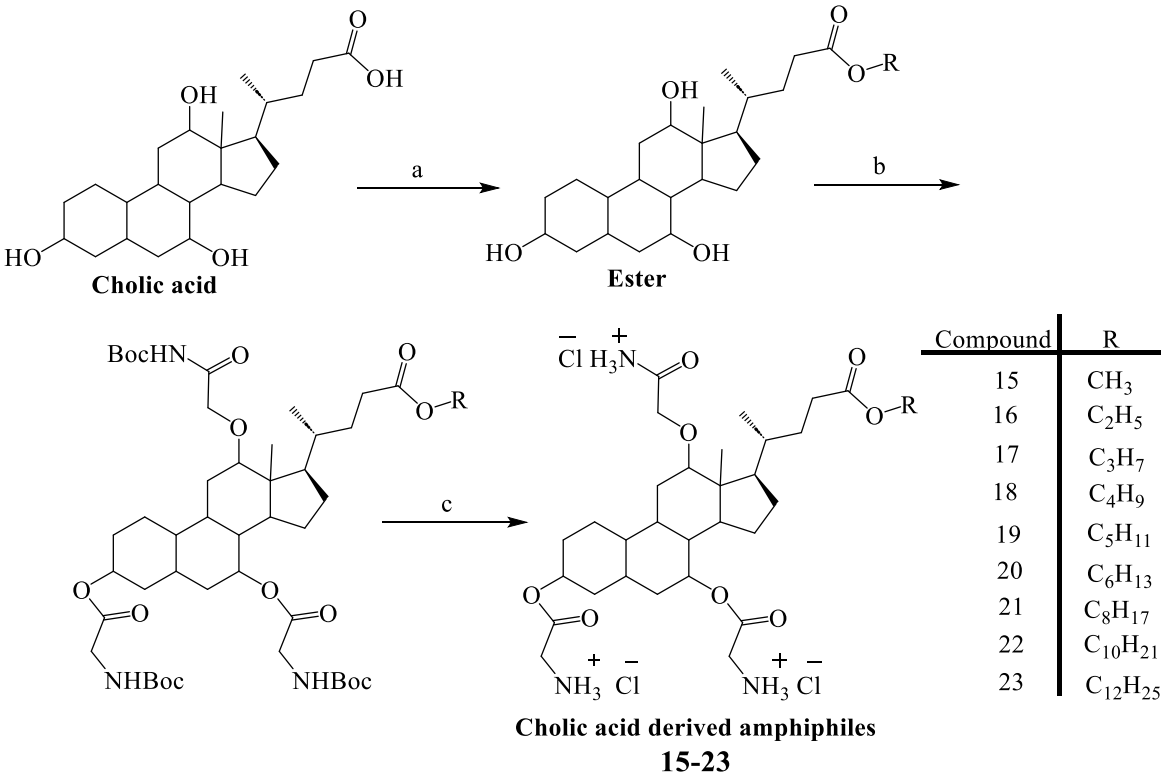
Several facial amphiphiles derived from cholic acid enhance membrane permeability, including that of bacterial cell walls [15]. These compounds contain nitrogen-based functionalities linked to hydroxyl groups, rendering bacteria more susceptible to various antibiotics. The positively charged groups interact with the bacterial membrane that has a negative charge. The attachment of eight carbon hydrophobic chain enhances antimicrobial activity by promoting self-facilitated transport across the bacterial outer membrane [27, 28]. In this context, Willeman et al. synthesized novel cholic acid-based facial amphiphiles having persistent ionic properties. These substances functioned as three-headed surfactants, creating larger secondary spherical micelles and smaller primary ones. The alkyl tail length influenced the micellar size [29]. This family of ionic facial amphiphiles was synthesized from cholic acid in three steps (Scheme 2). Initially, the lengthy alkyl chain was used to esterify the carboxylic group. Next, ω-bromoalkanoic chloride was used to introduce a spacer at each of the three hydroxyl groups. Finally, bromine atoms were replaced with trimethylamine, yielding trimethylammonium functional groups.



Scheme-2: a) $C_nH_{2n+1}OH$, p-toluenesulfonic acid, benzene, 908°C, 24 hours, 75%; b) Br-$(CH_2)_m$-COCl (ω-bromoalkanoic chloride), Dimethylaminopyridine, benzene, pyridine, Room temperature, 24 hours, 65%; c) Trimethylamine, Ethanol, 100°C, 24 hours, 90%.

The minimum inhibitory concentration (MIC) was evaluated against *Escherichia coli* and *Enterococcus faecalis*. Compounds **13** and **14** exhibited antibacterial activity by inhibiting bacterial growth, with MIC values of 25 µg/mL and 12.5 µg/mL, respectively. Notably, the antibacterial activity of compound **14** was comparable to that of chloramphenicol. In contrast, compounds bearing longer alkyl chains demonstrated no activity at concentrations up to 200 µg/mL.

Similarly, Yadav et al. synthesized cholic acid-based amphiphiles containing three glycine residues at C-3, C-7, and C-12, along with different hydrophobic alkyl chains at C-24 (Scheme 3). To introduce a net facial amphiphilic character, they incorporated three glycine residues as a cationic charge at the hydroxyl terminal and modified the cholic acid scaffold at the C-24 carboxylate with different hydrophobic alkyl chains. The synthesis of these compounds involved three key steps: (a) Esterification of the C-24 carboxylic acid using various alkyl alcohols. (b) Coupling of the three cholic acid hydroxyl termini with *Boc*-protected glycine units. (c) Removal of *Boc* protecting groups [30]. The antibacterial properties of these amphiphiles were assessed through the broth dilution assay, and minimum inhibitory concentration was calculated against various Gram-negative bacteria (Table 1).



Scheme-3: Synthesis of CA-derived amphiphiles. Reagents, reaction conditions, and yields: (a) ROH, HCl, 25 °C, 12 h (for a−d) or ROH, Dicyclohexylcarbodiimide, Dimethylaminopyridine, Dichloromethane (for e−i) 67−96%; (b) Boc-Gly-OH, DCC, DMAP, DCM, 25 °C, 12 h, 64−85%; (c) Dioxane-HCl, DCM, 0 °C, 3 h (for 15−20) or Trifluoroacetic acid, DCM, 0 °C, 3h followed by Amberlite 900 Cl− exchange resin (for compound 21−23) 78−95%.

Compound **20**, which contains a hexyl chain, exhibited the highest antibacterial activity, with an MIC_{99} of 4.0 μM. However, further elongation of the alkyl chain resulted in a decline in antibacterial activity [31]. A similar study was conducted by Singla et al., who investigated lysine-based cationic amphiphiles of cholic acid to evaluate their ability to penetrate bacterial membranes. They synthesized cholic acid–lysine cationic amphiphiles employing di-, tri-, and tetra-lysine conjugates at different functional regions to fine-tune cationic charges. The synthesized conjugates demonstrated broad-spectrum antibacterial activity towards *S. aureus*, *E. coli*, and *C. albicans*, both alone and in conjunction with known antimicrobial agents such as voriconazole and amphotericin B [32].

Table-1: Antibacterial activities of cholic acid derived amphiphiles.

Compounds	MIC_{99} (μM)					IC_{50} (μM)
	Gram-negative bacteria					
	S. Flexneri	*S. typhimurium*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	
15	**128**	**128**	**64**	**256**	**128**	**82**
16	**256**	**256**	**256**	**64**	**256**	**78**
17	**128**	**128**	**128**	**32**	**128**	**75**
18	**32**	**32**	**32**	**16**	**64**	**20**
19	**16**	**8**	**16**	**8**	**16**	**40**
20	**4**	**4**	**4**	**4**	**4**	**25**
21	**16**	**16**	**4**	**8**	**4**	**62**
22	**32**	**>256**	**32**	**16**	**8**	**23**
23	**>256**	**>256**	**>256**	**>256**	**>256**	**19**
Polymyxin B	**1**	**1**	**2**	**0.5**	**4**	**90**

Cholic acid peptide conjugates

Yadav et al. synthesized cholic acid–peptide conjugates (CAPs) and assessed their antibacterial activity [12] using the broth dilution method and determined minimum inhibitory concentration (MIC). They synthesized 20 CAPs with the general chemical formula CA−(G−X)$_3$, where X represents any naturally occurring amino acid conjugated to cholic acid via a glycine linker. The findings revealed that the most potent antibacterial agents were **CAP 25** and **CAP 26**, displaying MIC$_{99}$ of 8 µM (Table 2). CAP 25 and CAP 26 were derived from valine–glycine dipeptide and isoleucine dipeptide, respectively (Fig 3).

Table-2: Antibacterial activities of cholic acid peptide conjugates.

Compounds	MIC$_{99}$ (µM)			IC$_{50}$ (µM)
	Gram-negative bacteria			
	E. coli	*K. pneumoniae*	*A. baumannii*	
CAP 25	**8**	**16**	**16**	**56.42**
CAP 26	**8**	**16**	**16**	**7.68**
Polymyxin B	**2**	**4**	**0.5**	**Not determined**

Bile acid methylamine and ethylenediamine conjugates

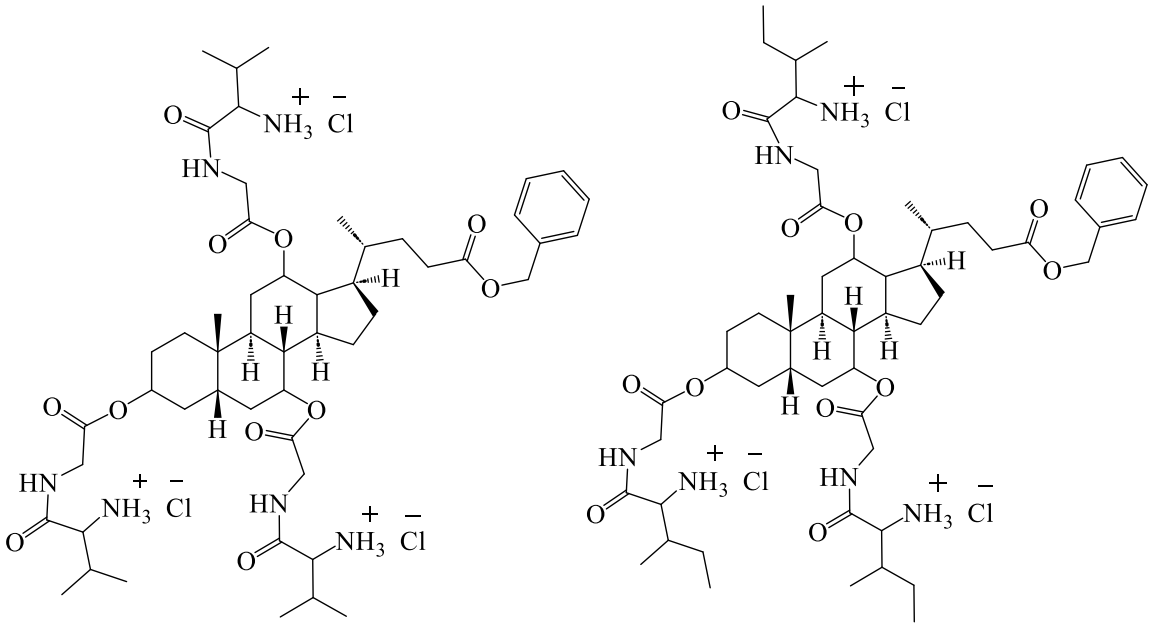
Aher et.al [33] synthesized bile acid–methylamine derivatives (**27**, **28**) and bile acid–ethylenediamine conjugates (**29**, **30**) to investigate the potential against the bacteria and fungi. In these compounds, the amino functionality was introduced one carbon away from the C-3 position of the steroid unit (Scheme 4). These amino sterols were synthesized using C-3β-oxiranes as essential intermediates. The potential of these compounds was assessed against various bacterial and fungal strains. Following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [34], the conventional broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) and IC$_{50}$ values. Standard antimicrobial agents, gentamicin and fluconazole, were used as reference drugs for comparison of antibacterial and antifungal efficacy, respectively.

Compounds **27**, **28**, **29**, and **30** demonstrated potent antibacterial and antifungal activity. Notably, compounds **29** and **30** exhibited activity comparable to or greater than gentamicin against *Staphylococcus aureus* (Table 3).

Table-3: Antibacterial activity of bile acid ethylenediamine conjugates.

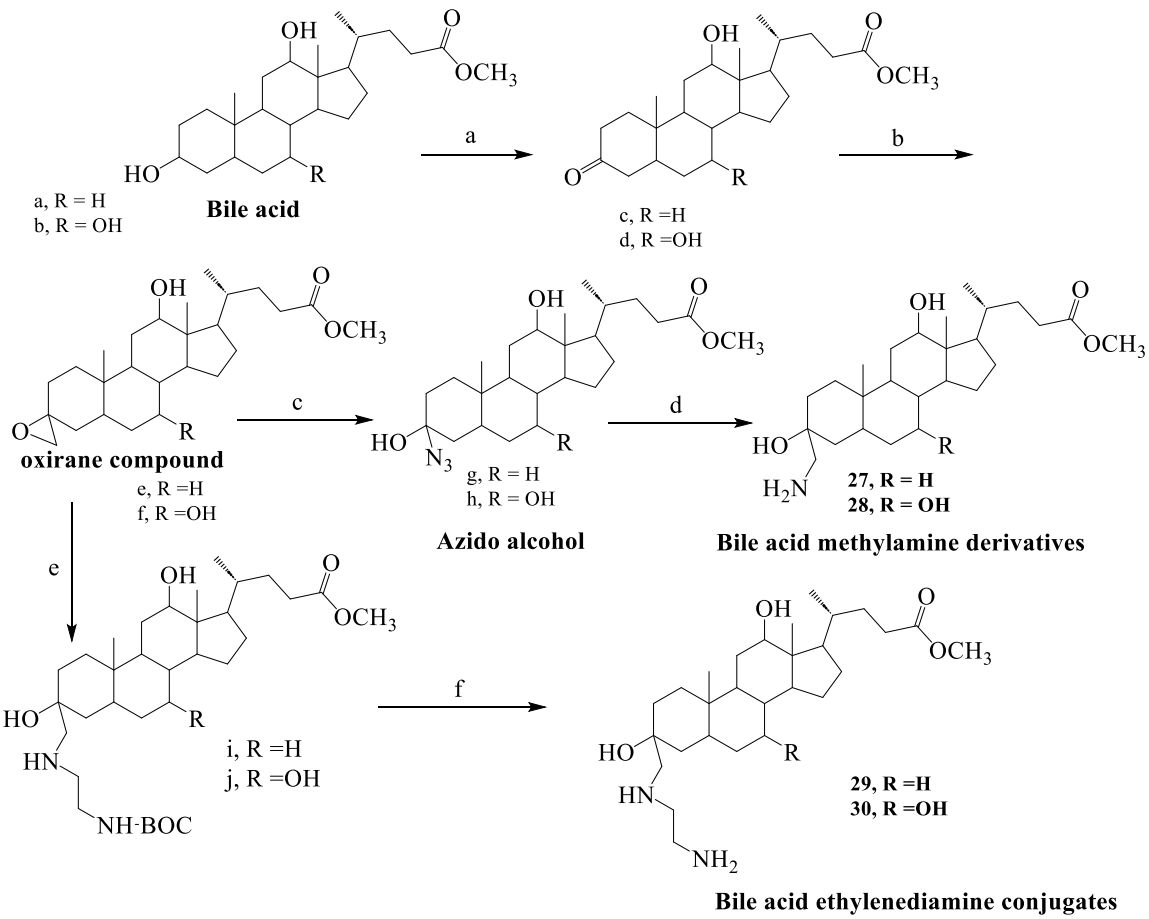
Compounds	Bacteria	IC$_{50}$ µg/mL	Ref.
29		**5.14**	**[33]**
30	***Staphylococcus aureus***	**4.12**	
gentamicin		**4.46**	

Compounds **29** and **30** exhibited activities against the tested fungi however, fluconazole demonstrated greater efficacy (Table 4).



Valine-glycine CAP 25 **Isoleucine CAP 26**

Fig. 3: Structure of potent CAPs.



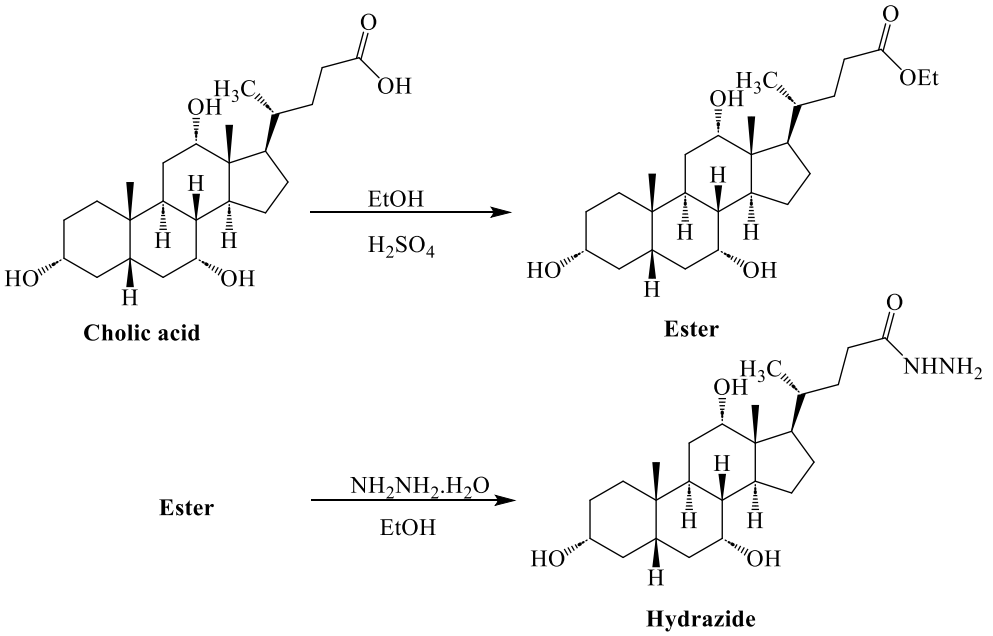
Scheme-4: Reagents and conditions: (a) Ag_2CO_3, toluene, reflux, 5 h; (b) trimethylsulfoxonium iodide, Sodium hydride, Dimethyl sulfoxide–Tetrahydrofuran, room temperature, 2 h; (c) NaN_3 (sodium azide), Dimethylformamide, 60–65 °C, 12 h; (d) H_2, Pd–C, MeOH, 45 psi, 3 h; (e) N_1-(Boc)-1,2-diaminoethane, MeOH reflux 2 h; (f) (i) 50% TFA/CH_2Cl_2; (ii) 50% DIPEA/CH_2Cl_2, overall in two steps.

Table-4: Antifungal activity of bile acid ethylenediamine conjugates.

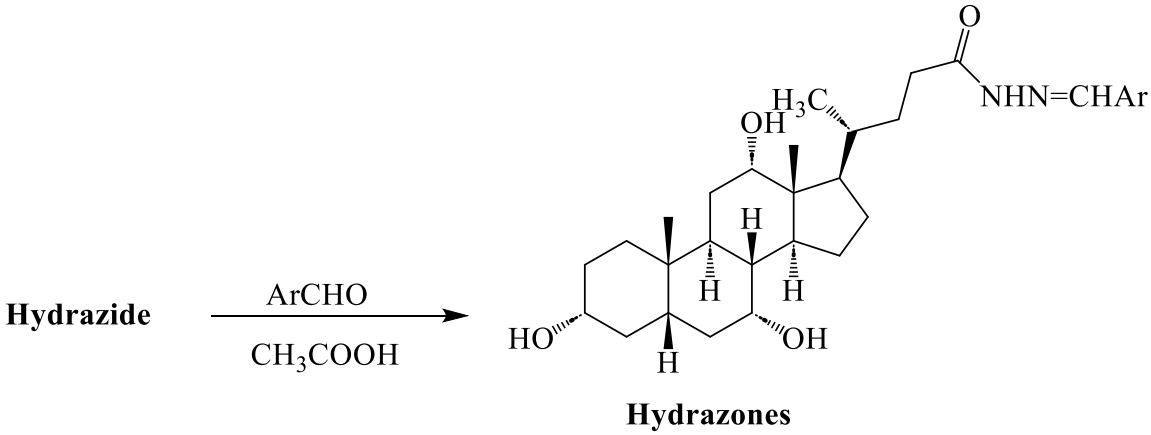
Comp	Fungi	IC_{50} (µg/mL)	Fluconazole IC_{50} (µg/mL)	Ref.
30	***Trichophyton mentagrophytes***	**6.25**	**0.6**	**[33]**
29	***Sporothrix schenckii***	**3.36**	**1.45**	
	Trichophyton mentagrophytes	**6.25**	**0.6**	
	Aspergillus fumigatus	**8.14**	**1.06**	

Cholic acid-based hydrazone derivatives

The extensive and excessive use of antibacterial drugs has led to increased bacterial resistance to commonly used antibiotics. Consequently, there is now significant research focused on developing novel antibiotics [26, 35]. In this context, Rasras et al. [36] evaluated the cholic acid-based hydrazone analogs against the bacteria. The conversion of cholic acid to its ester form is achieved using methanol as a solvent, with concentrated H_2SO_4 acting as a dehydrating agent. Hydrazides are then formed by transforming the corresponding esters in the presence of hydrazine under reflux conditions (Scheme 5). The hydrazide is subsequently converted into the desired hydrazone compound by introducing an aldehyde or ketone into the reaction via reflux (Scheme 6).



Scheme-5: Synthesis of cholyl hydrazide.



Scheme-6: Synthesis of hydrazone (**31-51**).

The antibacterial activity of the synthesized compounds was assessed against both Gram-positive and Gram-negative bacterial strains. Minimum inhibitory concentrations (MICs) were determined for all compounds. Overall, the compounds exhibited notable antibacterial efficacy against several tested strains; however, they showed no activity against *Enterobacter aerogenes* and *Pseudomonas aeruginosa*.

Compared to the gram-positive bacterial controls, cefaclor and cefixime (MIC 31.25 µg/mL), compounds **33**, **39**, and **40** demonstrated potent activity against *Enterococcus faecalis* with an MIC of 1.96 µg/mL, making them fifteen times more effective (Fig 4). Similarly, compounds **34**, **44**, and **45** exhibited eight times the activity of the controls against the same bacterial species, with an MIC of 3.91 µg/mL. Against *B. megaterium*, most tested compounds displayed MIC values comparable to cefaclor, except for compounds **34**, **45**, and **48**, which were more effective having minimum inhibitory concentration of 7.82, 15.63, and 15.63 µg/mL, respectively (Table 5).

Table-5: Antibacterial activity of the synthesized hydrazones.

Compound	MIC (µg/mL)					
	Gram-negative			Gram-positive		
	E. coli	*P. aeruginosa*	*E. aerogenes*	*S. aureus*	*E. faecalis*	*B. megaterium*
31	**3.91**	**na**	**na**	**62.5**	**15.63**	**31.25**
32	**na**	**na**	**na**	**31.25**	**62.5**	**31.25**
33	**3.91**	**na**	**na**	**62.5**	**1.96**	**31.25**
34	**3.91**	**na**	**na**	**62.5**	**3.91**	**7.82**
35	**7.81**	**na**	**na**	**31.25**	**7.82**	**na**
36	**7.81**	**na**	**na**	**62.5**	**15.63**	**31.25**
37	**7.81**	**na**	**na**	**31.25**	**15.63**	**31.25**
38	**7.81**	**na**	**na**	**31.25**	**7.82**	**31.25**
39	**3.91**	**na**	**na**	**31.25**	**1.96**	**31.25**
40	**7.81**	**na**	**na**	**na**	**1.96**	**31.25**
41	**na**	**na**	**na**	**31.25**	**7.82**	**31.25**
42	**3.91**	**na**	**na**	**na**	**15.63**	**31.25**
43	**na**	**na**	**na**	**na**	**15.63**	**31.25**
44	**7.81**	**na**	**na**	**31.25**	**3.91**	**31.25**
45	**na**	**na**	**na**	**31.25**	**3.91**	**15.63**
46	**na**	**na**	**na**	**na**	**31.25**	**na**
47	**na**	**na**	**na**	**na**	**na**	**31.25**
48	**na**	**na**	**na**	**62.5**	**31.25**	**15.63**
49	**na**	**na**	**na**	**na**	**na**	**31.25**
50	**7.81**	**na**	**na**	**na**	**na**	**31.25**
51	**na**	**na**	**na**	**na**	**31.25**	**31.25**
Cefaclor	**na**	**na**	**7.82**	**31.25**	**31.25**	**31.25**
Cefixime	**1.96**	**19.54**	**31.25**	**31.25**	**31.25**	**na**

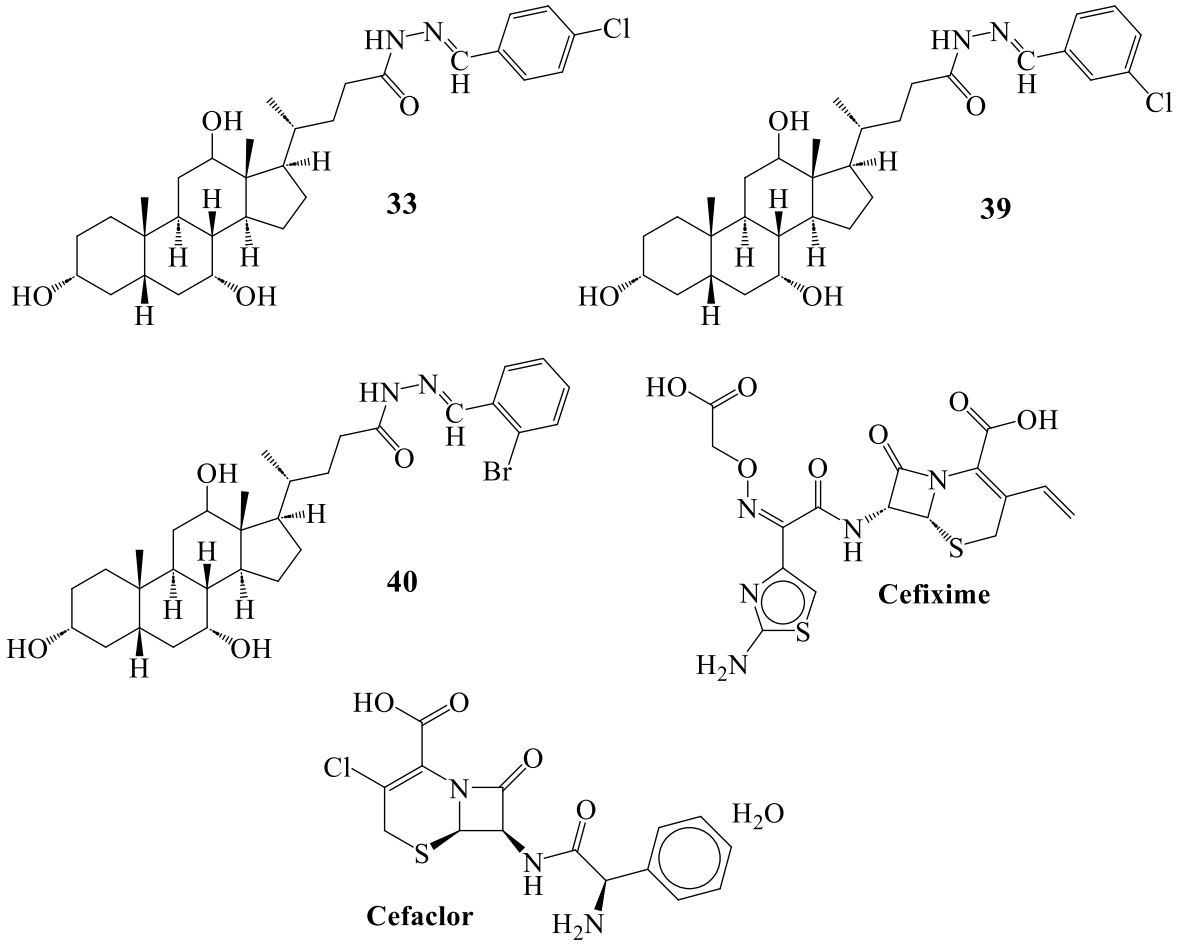


Fig. 4: Structures of cholic acid-based hydrazones alongwith standards.

Regarding gram-negative bacterial controls, several derivatives displayed antibacterial activity comparable to or lower than that of cefaclor and cefixime. Compounds **31**, **33**, **34**, **40**, and **42** were found to be twice as effective as cefixime against *E. coli* (MIC 3.91 µg/mL) and demonstrated greater potency than cholic acid itself. Notably, *S. aureus* is a highly adaptable human opportunistic pathogen responsible for numerous infections and fatalities worldwide [37]. The growth of these bacteria can also be inhibited by increasing membrane disruption and leakage of cellular contents, leading to bacteriostatic effects, as observed with unconjugated cholic acid derivatives exhibiting an MIC of 20 mM [38].

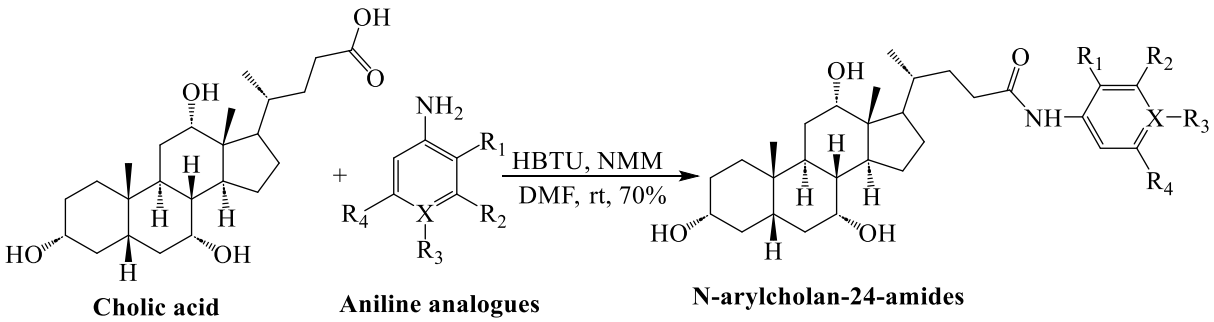
Organometallic complexes of cholic acid

Cholic acid has been identified as a promising lead molecule for developing organometallic complexes with enhanced antibacterial and antifungal activity, owing to the synergistic effects of metal ions and cholic acid [39]. To investigate this, Kishu et al. examined the antibacterial properties of organometallic complexes of cholic acid against gram-positive and gram-negative bacterial strains. The zone of inhibition around the discs was measured to determine antibacterial efficacy. Standard antibiotic discs containing 10 µg of gentamycin and 10 µg of CA acted as positive controls. The inhibition zone diameters were recorded in millimeters, and each test was performed three times to minimize experimental error. All the compounds displayed antibacterial potential against respective bacteria. Many compounds showed greater activity than the standard antibiotic gentamycin and the cholic acid activity. Some of the compounds displayed equal potent activity to the standard. In case of gram-positive *Streptococcus pneumonia*, none of the compounds displayed greater potential than cholic acid as antibacterial. Similarly, in case of gram-negative *Klebsiella pneumonia* only one compound showed equal potent activity as of cholic acid as antibacterial, while remaining compounds were less active than cholic acid [40].

Cholic acid-based amide analogues

Clostridium difficile infections (CDI) pose significant treatment challenges [41], particularly when involving the hypervirulent BI/NAP1/027 pandemic strains [42]. CDI treatment typically requires a strict regimen combining antibiotic therapy with rigorous environmental decontamination measures [43]. However, complete eradication remains difficult because of *Clostridium difficile*'s capability to develop resilient spores [44]. Previously, cholic amide *m*-sulfonic acid (CamSA) effectively inhibited spores from germinating, but CamSA has proven ineffective against the hypervirulent strain R20291. In response, Sharma et al. [45] generated and tested a number of cholic acid amides specifically targeting gram-positive bacteria, *Clostridium difficile* R20291 (Scheme 7).

A series of *N*-arylcholan-24-amides (**52a−n**) were synthesized by pre-activating cholic acid with HBTU/NMM in DMF at room temperature, followed by in situ reaction with various substituted or unsubstituted aniline analogues. Among these, the simple phenyl amide analogue **52a** (Fig 5) exhibited the highest potency, with an IC_{50} value of 1.8 µM. Notably, **52a** demonstrated over 225 times greater activity than chenodeoxycholate, a natural germination inhibitor.



Scheme-7: Synthesis of amide analogues. Reagents and conditions: HBTU (1H-benzotriazol tetramethyluronium hexafluorophosphate), NMM (N-methylmorpholine), DMF (Dimethyl formamide).

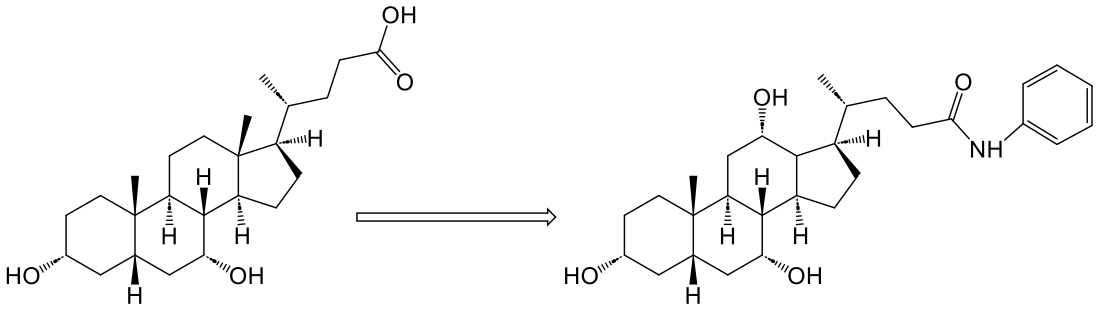


Fig. 5: Chenodeoxycholic acid and phenyl amide cholic acid analogue.

Cholic acid derived amino α-cyanostilbene derivatives

Amino α-cyanostilbene-based compounds exhibit potent antibacterial activity [46]. These compounds possess fluorescent properties, enabling them to stain bacteria, which may facilitate their penetration into bacterial cells. To explore this potential, Agarwal et al. synthesized bile acid–amino-substituted α-cyanostilbene conjugates and evaluated their antibacterial activity against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacterial strains. These conjugates demonstrated enhanced antibacterial activity compared to their corresponding amides. Among them, the cholic acid–furan-derived amino α-cyanostilbene derivative **53** (Fig 6) exhibited superior antibacterial efficacy, surpassing the standard antibiotic chloramphenicol. The IC_{50} values for these compounds are presented in Table 6.

Cholic acid analogues from Trinidad pitch lake

Dobson et al. [47] isolated six cholic acid analogues (Fig 7) from *Bacillus amyloliquefaciens* UWI-W23, a strain isolated from Trinidad Pitch Lake. Among these, taurodeoxycholate **(55)** was reported initially from a bacterial source, however deoxycholate **(57)** was identified initially from a Gram-positive bacterium. Remaining compounds **(54)**, **(56)**, **(58)**, and **(59)**—had not been previously identified in *Bacillus* species.

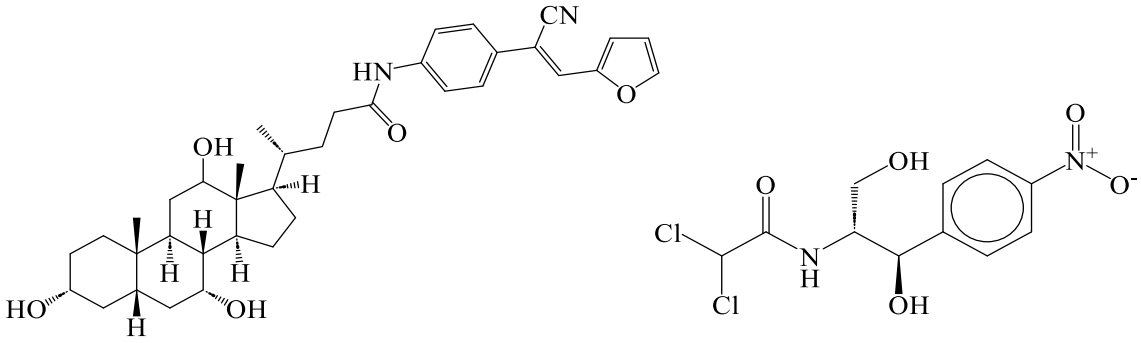
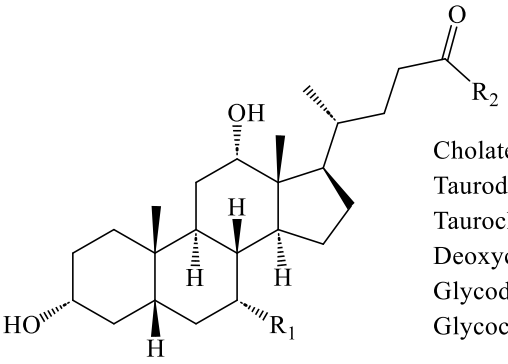


Fig. 6: Structure of cholic acid furan derivatized amino α-cyanostilbene and chloramphenicol.

Table-6: Antibacterial activity of Cholic acid furan derivatized amino α-cyanostilbene.

Compound	Antibacterial activity				Ref.
	Gram positive (IC_{50} µg/mL)		Gram negative (IC_{50} µg/mL)		
	B. subtilis	***S. aureus***	***E. coli***	***S. typhi***	
Cholic acid furan derivatized amino α-cyanostilbene	**16**	**>8**	**>8**	**>8**	**[46]**
Chloramphenicol	**16**	**16**	**16**	**32**	



Cholate	54	R_1=OH	R_2=O^-
Taurodeoxycholate	55	R_1=H	R_2=NH-$(CH_2)_2$-SO_3^-
Taurocholic acid	56	R_1=OH	R_2=NH-$(CH_2)_2$-SO_3H
Deoxycholate	57	R_1=H	R_2=O^-
Glycodeoxycholic acid	58	R_1=H	R_2=NH-(CH_2)-COOH
Glycocholic acid	59	R_1=OH	R_2=NH-(CH_2)-COOH

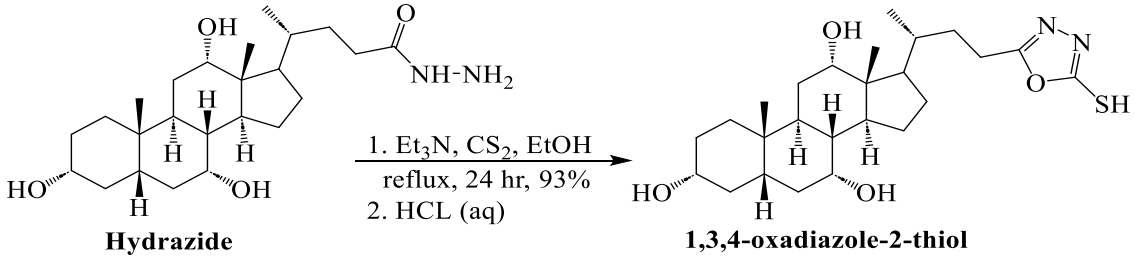
Fig. 7: Cholic acid analogues from Trinidad pitch lake.

All compounds exhibited antibacterial activity, with MIC values ranging from 7 to 250 µg/mL against gram-positive and gram-negative bacteria. Additionally, glycocholic acid **(59)** demonstrated efficacy against *Saccharomyces cerevisiae* (MIC = 15.6 µg/mL), while cholate **(54)** was effective against MRSA showing MIC of 125 µg/mL. In the context of combating multidrug-resistant bacteria, several studies have explored synergistic drug combinations [48, 49]. Rashid et al. conjugated cholic acid with ampicillin to form CA-AMP **(60)** and evaluated its antibacterial activity against β-lactamase-producing bacteria. CA-AMP exhibited a synergistic effect, showing increased antibacterial activity against all tested microorganisms, with a low fractional inhibitory concentration index (FIC ≤ 0.5) [50].

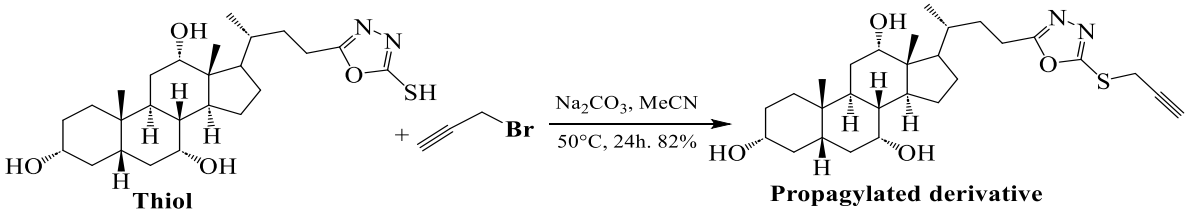
Cholic acid-based oxadiazole hybrids

Rasras et al. [51] synthesized a novel library of cholic acid analogues hybridized with the heterocyclic 1,3,4-oxadiazole unit and assessed their antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungi. Cholic acid was initially transformed into cholylhydrazide [36]. The 1,3,4-oxadiazole unit was then introduced through a reaction with carbon disulfide and trimethylamine under reflux in ethanol, yielding 1,3,4-oxadiazole-2-thiol with a 93% yield (Scheme 8) [52].

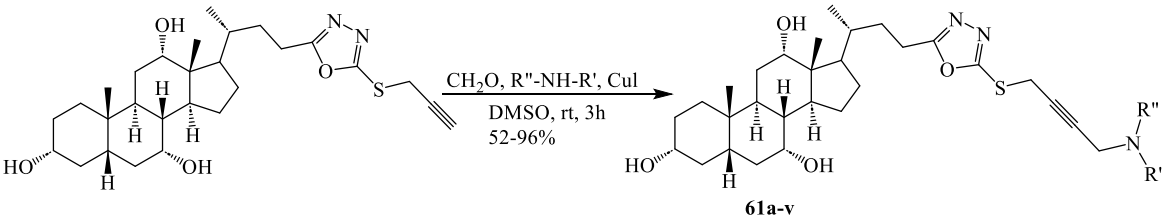
The thiol product was subsequently reacted with propargyl bromide and Na_2CO_3, yielding the thiopropagylated derivative with an 82% yield after 24 hours (Scheme 9) [52].



Scheme-8: Synthesis of cholyl 1, 3, 4-oxadiazole-2-thiol.



Scheme-9: Synthesis of cholyl 2-(propargylthio)-1, 3, 4-oxadiazole.



Scheme-10: Synthesis of target compounds **(61a-v)**.

The propargylated derivative was then converted into the desired compounds by reacting its alkyne group with formaldehyde in the presence of a R_2NH and Copper iodide as a catalyst in DMSO, with stirring at room temperature for 3 hours (Scheme 10) [53, 54].

Among the synthesized compounds (61a-v), 61t, 61i, 61p, 61c, and 61d were the most active compounds (Fig 8). 61t was the most active compound against gram-positive *S. aureus* having zone of inhibition of 36.2 ± 1.9 mm surpassing the standard gentamycin. The compound 61d was the most active against gram-negative *P. vulgaris* having zone of inhibition of 22.3 ± 1.7 mm but less active than the standard gentamycin. Overall, these compounds showed good in vitro antibacterial and antifungal activities (Table 7).

Table-7: Antimicrobial activity of cholic acid oxadiazole hybrids.

Compounds	Zone of inhibition (mm)					
	Fungi		Gram-positive bacteria		Gram-negative bacteria	
	C. albicans	*A. fumigatus*	*S. aureus*	*B. subtilis*	*E. coli*	*P. vulgaris*
61t	**13.4 ± 1.5**	**13.1 ± 1.3**	**36.2 ± 1.9**	**19.1 ± 0.7**	**14.2 ± 0.9**	**20.9 ± 1.1**
61i	**18.9 ± 1.2**	**18.3 ± 1.5**	**35.3 ± 1.9**	**24.3 ± 1.7**	**15.1 ± 0.5**	**9.4 ± 1.2**
61p	**16.7 ± 1.3**	**16.2 ± 1.4**	**33.5 ± 1.9**	**26.7 ± 1.8**	**15.4 ± 1.2**	**11.7 ± 0.8**
61c	**9.1 ± 0.7**	**10.2 ± 0.8**	**33.4 ± 1.2**	**19.1 ± 1.3**	**17.8 ± 0.9**	**21.2 ± 1.6**
61d	**11.9 ± 1.1**	**10.8 ± 0.6**	**25.1 ± 0.8**	**17.5 ± 1.4**	**15.2 ± 0.9**	**22.3 ± 1.7**
Ketoconazole	**25.7 ± 1.5**	**26.2 ± 1.6**	**-**	**-**	**-**	**-**
Gentamycin	**-**	**-**	**31.9 ± 1.7**	**33.1 ± 1.9**	**29.5 ± 1.3**	**28.8 ± 1.6**

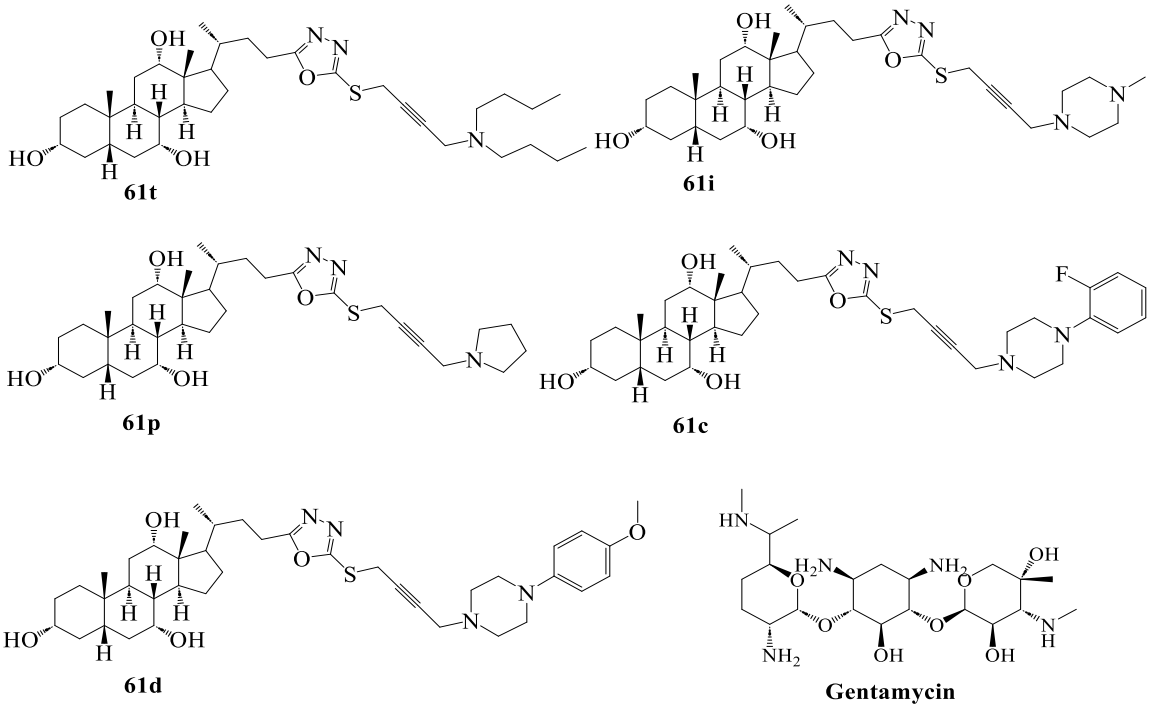
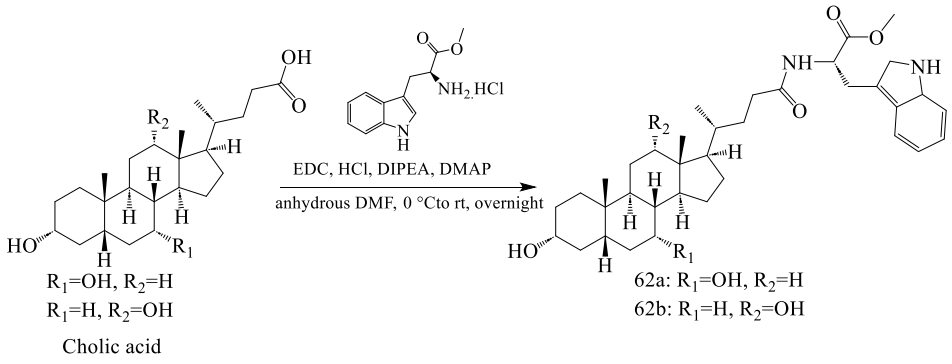


Fig. 8: Effective compounds structure [51].

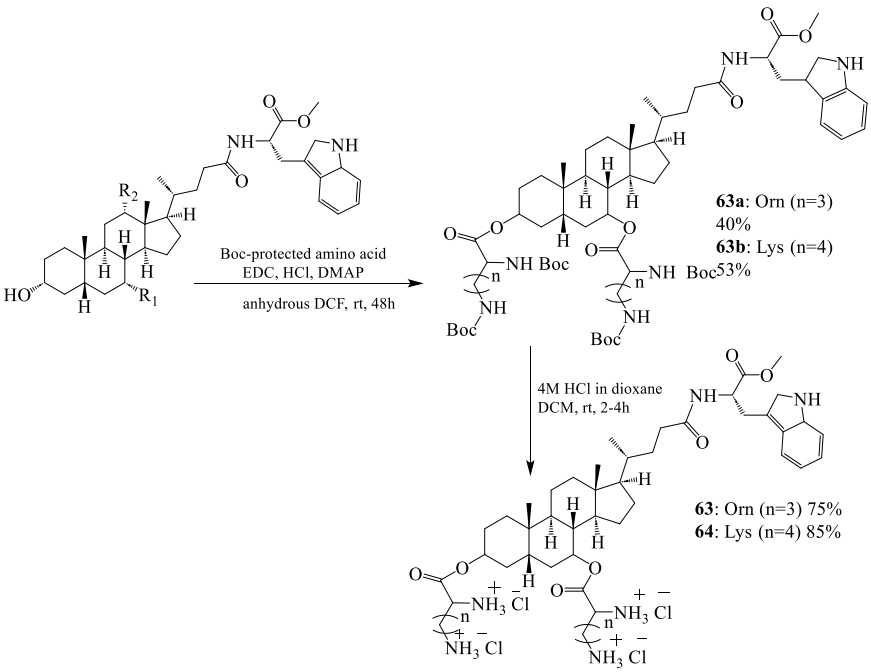
Cholic acid-based antimicrobial peptides

Antimicrobial peptides found in nature are essential in the defense mechanisms of organisms, particularly humans. These peptides exhibit potent antimicrobial activity by directly targeting bacterial cell membranes, disrupting membrane permeability, and ultimately leading to bacterial cell death [55, 56]. Cholic acid-based antimicrobial peptides have also demonstrated significant antibacterial activity [57]. Wu et al. [58] synthesized and evaluated the antimicrobial potential of cholic acid-based AMPs. In their approach, a hydrophobic moiety was attached to the carboxyl end of the cholic acid to generate new analogues. Then they added 1-3 amino acid residues to the OH-groups of cholic acid (Scheme 11).

SAR analysis indicated that the tryptophan unit was necessary for activity against microbes. The findings indicated that compounds **63** and **64**, containing ornithine and lysine as peptide units linked to the hydroxyl groups of cholic acid, exhibited the highest potential towards both Gram-positive and Gram-negative bacteria (Scheme 12). The compounds likely function as antibacterial pore-forming agents, as they demonstrated the ability to depolarize the bacterial membrane.



Scheme-11: Synthesis of tryptophan methyl ester substituted cholic acid derivatives. Reagents: EDC (Ethyl (dimethylaminopropyl) carbodiimide, DIPEA (Diisopropylethylamine), DMAP (Dimethylaminopyridine).

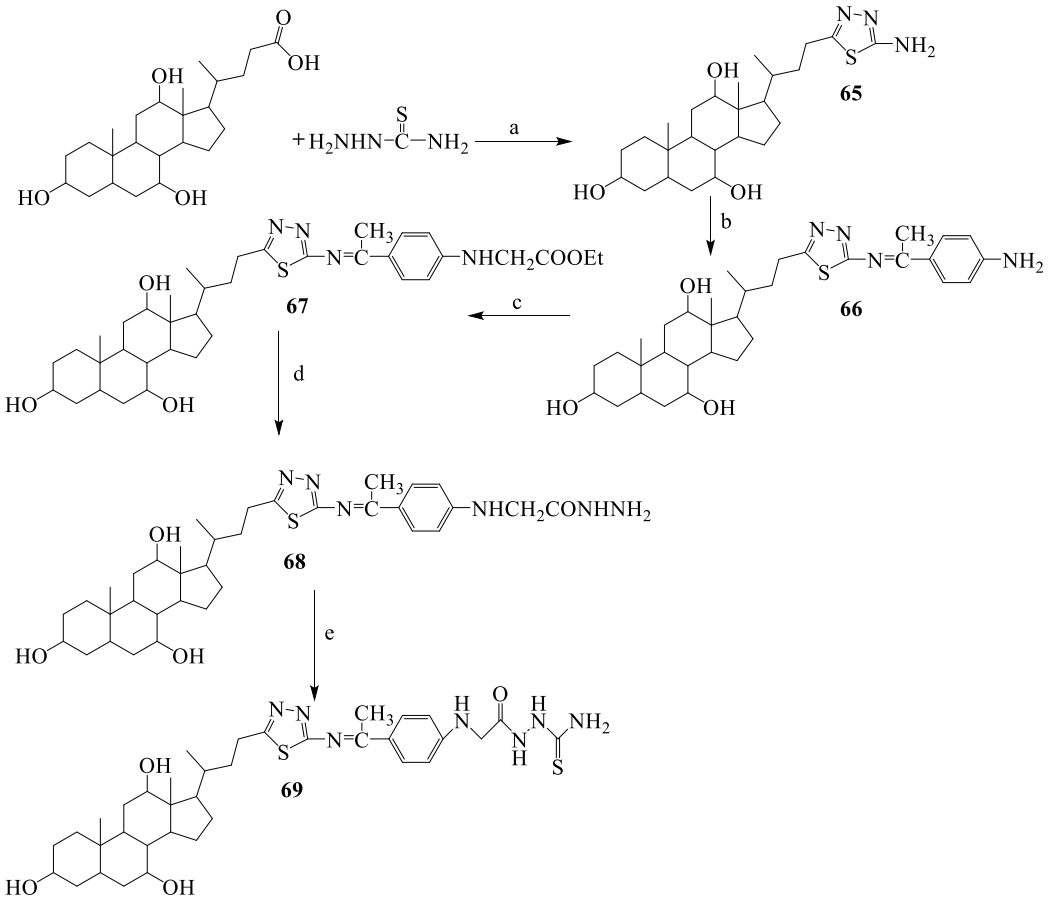


Scheme-12: Synthesis of the most active cholic acid-based AMPs.

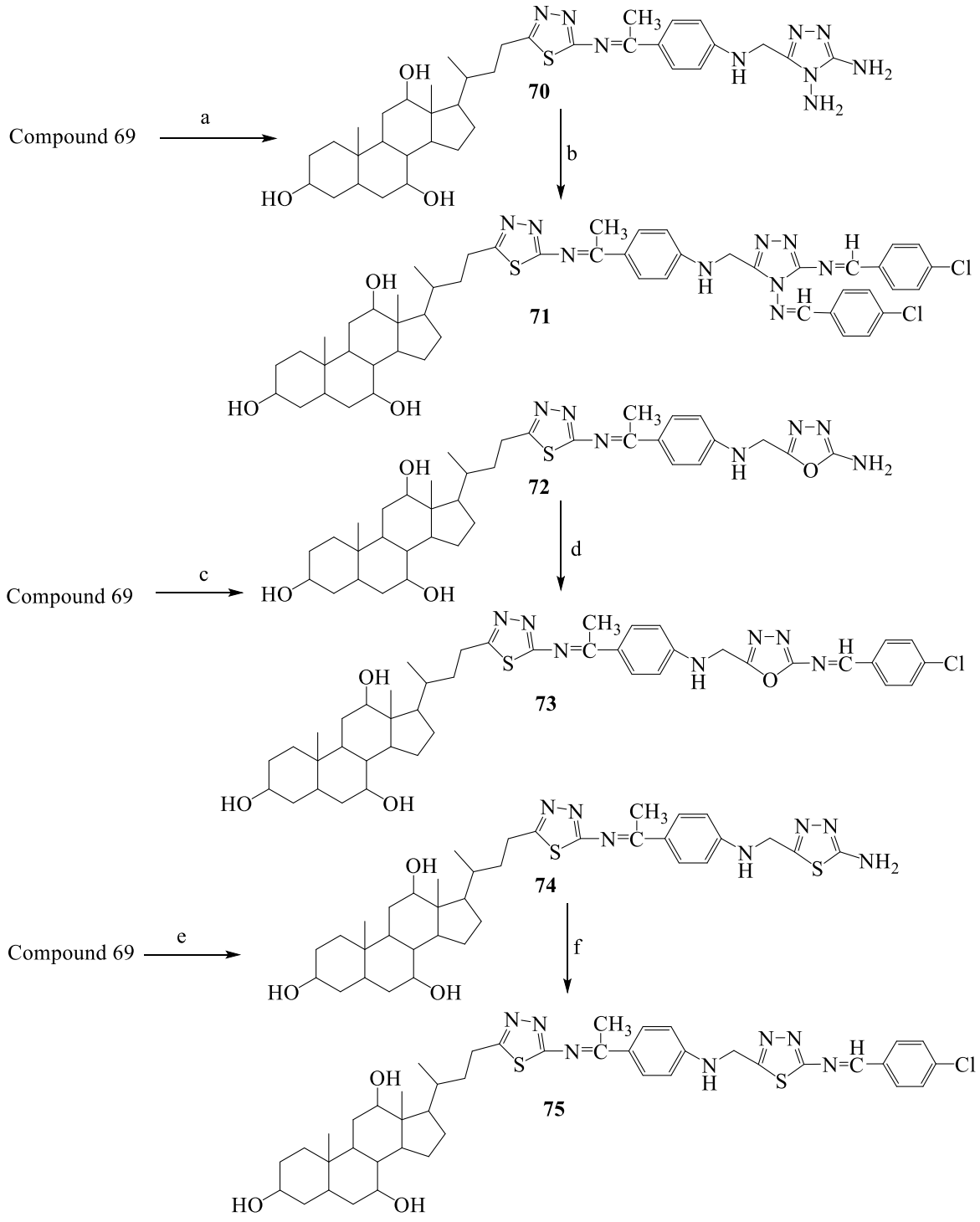
Cholic acid derived thiadiazole derivatives

Recently, Al-Araj et al. generated cholic acid analogues evaluating their antibacterial activity. Initially, an intermediate compound, substituted thiosemicarbazide, was synthesized along with several other compounds, and their antimicrobial properties were assessed. The synthesis began with cholic acid reacting with thiosemicarbazide to yield 2-choly-5-amino-1,3,4-thiadiazole **(65)**. Hydrazone **(66)** was then obtained by treating compound **65** with 4-aminoacetophenone and concentrated HCl in ethanol. Further reaction of hydrazone with ethyl 2-bromoacetate produced ester **(67)**, which was converted into acid hydrazide **(68)** using hydrazine hydrate in ethanol. The intermediate substituted thiosemicarbazide **(69)** was then synthesized by reacting compound **68** with ammonium thiocyanate (Scheme 13) [59]. Compound **69** was further transformed into 1,2,4-triazole (70), 1,3,4-oxadiazole (72), and 1,3,4-thiadiazole (74) through reactions with $N_2H_4.H_2O$, HgO, and conc. H_2SO_4, respectively. The Schiff bases **71**, **73**, and **75** were subsequently prepared by reacting compounds **70**, **72**, and **74** with 4-chlorobenzaldehyde and conc. HCl in ethanol (Scheme 14) [59].

The synthesized compounds demonstrated significant antimicrobial activity. Compounds **66**, **69**, and **71** displayed inhibition zone of 15 mm, 20 mm, and 19 mm, respectively. Notably, compound **75** showed the highest inhibition against *S. aureus* (23 mm) at 200 mg/mL, followed by compound **73** (21 mm). Against *P. aeruginosa*, compounds **69**, **72**, and **75** displayed zone of inhibition of 21 mm, 20 mm, and 18 mm, respectively, at the same concentration. Additionally, compounds **71** and **66** showed inhibition zones of 16 mm and 14 mm, respectively [59].



Scheme-13: Preparation of substituted thiosemicarbazide (69). Reagents and conditions:(a) Cholic acid, conc. H_2SO_4, steam bath (b) EtOH, 4-aminoacetophenone, refl. (c) abs. EtOH, bromo ethyl acetate, $NaHCO_3$, refl (d) $N_2H_4.H_2O$ 85% abs.EtOH, refl (e) ammonium thiocyanate abs. EtOH. HCl, refl.



Scheme-14: Preparation of compounds (70-75). Reagents and conditions: (a) $N_2H_4.H_2O$ 85% refl., (b) EtOH, glacial acetic acid, chlorobenzaldehyde, refl.; (c) HgO, MeOH, reflux (d) EtOH, glacial acetic acid, chlorobenzaldehyde, reflux; (e) conc. H_2SO_4,90 °C (f) EtOH, glacial acetic acid, chlorobenzaldehyde, refl.

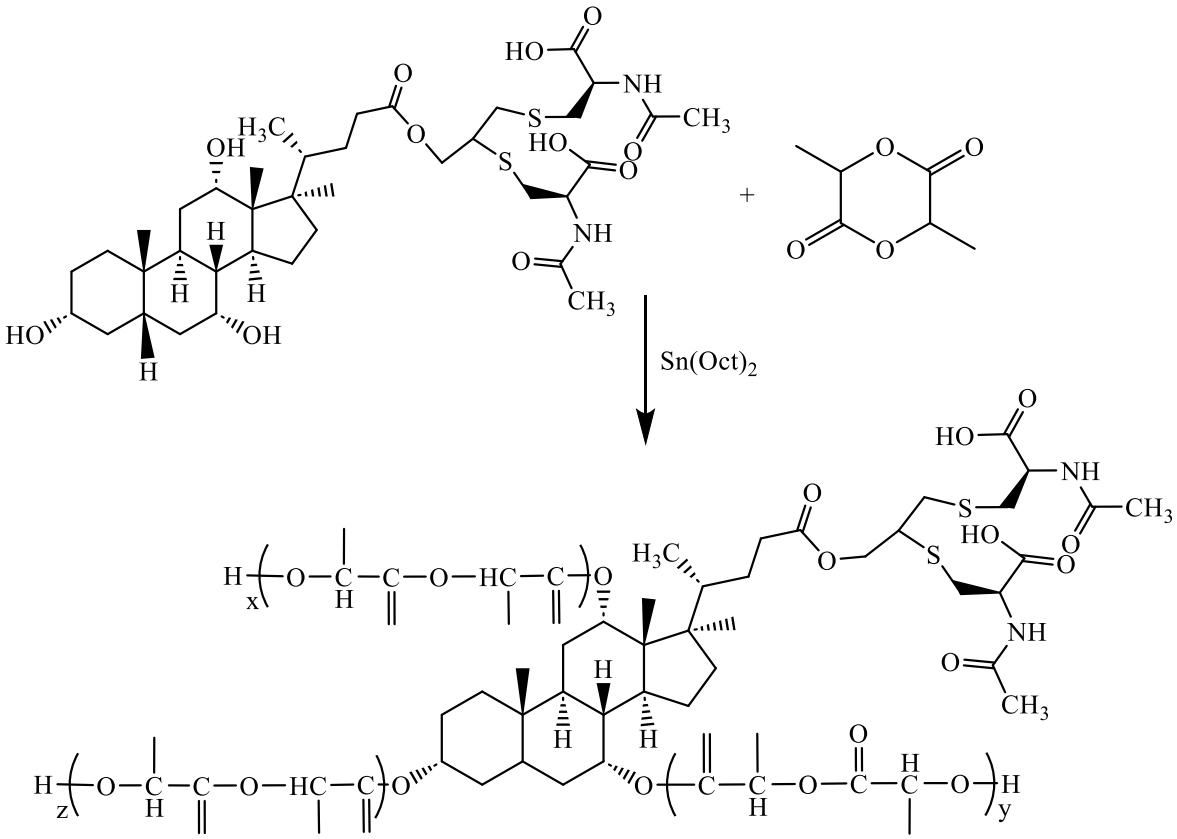
Similarly, Alshawabkeh et al. [60] produced oxadiazole analogues of cholic acid as well as evaluated their antibacterial efficacy by calculating MIC against both Gram-positive and Gram-negative bacteria. Several compounds exhibited potent antibacterial activity against *S. aureus* and methicillin-resistant *Staphylococcus epidermidis* (MRSE) with improved MIC values.

N-acetylcysteine-functionalized cholic acid-based triarmed poly(D,L-lactide)

Ezhumala et al. [61] synthesized N-acetylcysteine-functionalized cholic acid-based triarmed poly(D,L-lactide) as reverse polymeric micelles for drug delivery applications and assessed their antibacterial potential. The thiol-yne click reaction was utilized to synthesize ACyCA, utilizing (Sn (Oct)$_2$) as a catalyst (Scheme 15).

Spectrofluorometer was used for the determination of the reverse critical micellar concentration (RCMC) of the polymer, which was found to be 1.99 mg/mL. The synthesized reverse micelles (RMs) served as both capping and reducing agents during the production of gold nanoparticles in the presence of sunlight. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) analysis showed that the produced AuNPs were round in shape, having an average size of approximately 23.4 nm and a hydrodynamic diameter (D_h) of 86.8 ± 1.3 nm.

The antibacterial potential of the synthesized reverse micelles and RMs-AuNPs was evaluated using the well-diffusion method in Mueller-Hinton Agar (MHA) medium. Their MIC was calculated against various Gram-positive and Gram-negative bacterial strains, as well as fungal pathogens. The MIC values for *Enterococcus faecalis* were 256 µg/mL for RMs and 128 µg/mL for RMs-AuNPs. In comparison, the positive control exhibited much less MIC values: 1 µg/mL for *Staphylococcus aureus*, 32 µg/mL for *Escherichia coli*, 16 µg/mL for *E. faecalis*, and 4 µg/mL for *Pseudomonas aeruginosa*. However, RMs and RMs-AuNPs did not exhibit any MIC activity against *Candida albicans, P. aeruginosa* and *E. coli* [61].

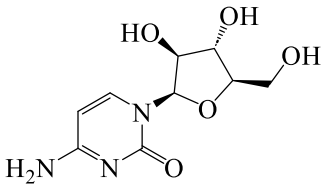


Scheme-15: Synthesis of ACyCA-triarmed (DLL)n **(76)**.

Anticancer activities

Cholic acid cytarabine conjugates against HL60 cells

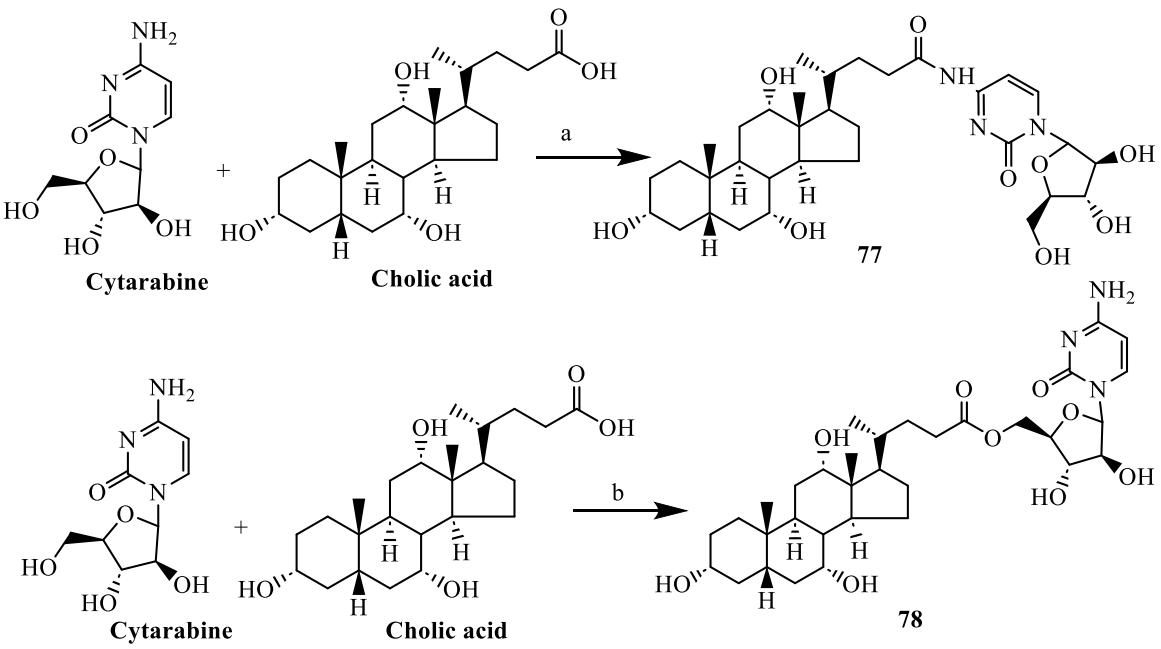
Cytarabine is a pyrimidine nucleoside analogue extensively utilized in the treatment of acute and chronic myeloblastic leukemias [62, 63], Hodgkin's lymphoma, and meningeal leukemia [64]. It exerts cytotoxic activity in its triphosphate form [65].



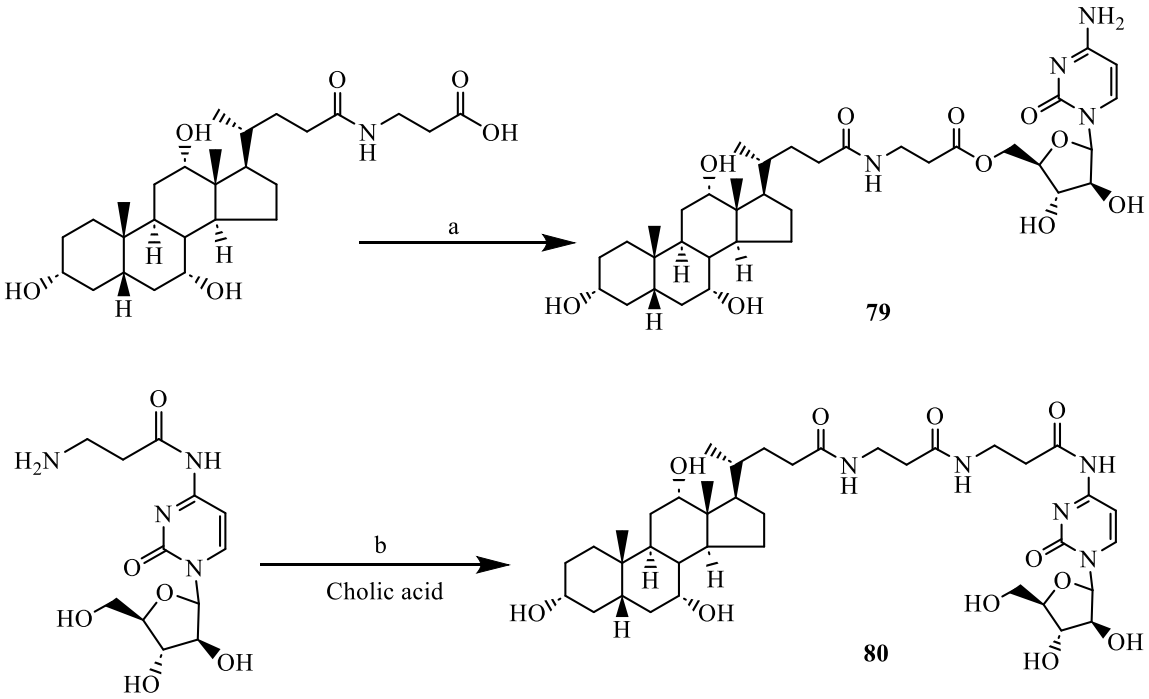
Cytarabine

However, due to its rapid metabolism, prodrugs of cytarabine have been developed to improve its pharmacokinetic properties [66]. To enhance cytarabine's pharmacokinetics and anticancer efficacy, Chen et al. [67] synthesized novel cholic acid–cytarabine conjugates and evaluated their anticancer activity against HL60 leukemia cells. Four different linkers were used to form cytarabine–cholic acid conjugates. In conjugate **77**, cytarabine and cholic acid were linked by a direct amide bond using ethyl chloroformate, whereas in conjugate **78**, they were connected through a direct ester bond (Scheme 16).

In derivatives **79**, **80**, β-alanine was utilized to connect cholic acid with cytarabine. The carboxyl group of β-alanine was connected to cytarabine via either an -CONH linkage (conjugate **79**) or an ester linkage (conjugate **80**). Meanwhile, the N-terminal of β-alanine was linked to cholic acid through an -CONH linkage, ensuring a stable conjugation (Scheme 17).



Scheme-16: (a) $ClCOOC_2H_5$, 272-rimethylamine, N,N-DMF, -15 °C to room temperature; (b) DCC, 4-DMAP, N,N-DMF.



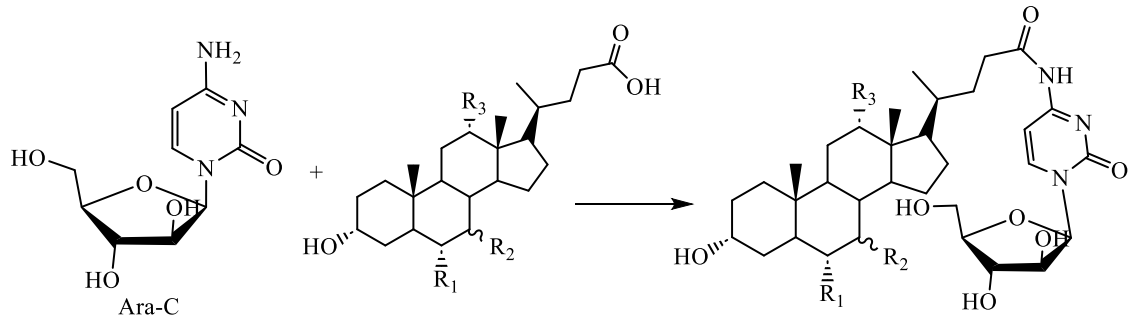
Scheme-17: (a) DCC, 4-DMAP, N,N-DMF; (b) $ClCOOC_2H_5$, TMA, N,N-DMF, -15 °C to room temperature.

Table-8: Antitumor IC_{50} of synthesized compounds.

Compounds	HL60 (IC_{50} (μmol/L))	Ref.
Cytarabine	**0.19 ±0.13**	**[67]**
77	**2.09±1.23**	
79	**0.99±0.65**	
80	**2.96±2.03**	

Conjugates **77** and **80** exhibited strong *in vivo* absorption and remarkable liver-targeting specificity, with liver target indices of 34.9 and 16.3, respectively, compared to cytarabine. Additionally, conjugates **77**, **79**, and **80** retained potent anticancer activity in HL60 cells sensitive to cytarabine (Table 8) [67].

In another study, Zhang et al. synthesized four cytarabine conjugates **(81–84)** and evaluated their biological activity. The BA-Ara-C conjugates were synthesized via a condensation reaction between the carboxyl group at the C-24 position of bile acids and the 4-amino group (4-NH_2) of cytarabine (Scheme 18).



CA: R_1=H R_2=OH, R_3=OH
CDCA: R_1=H R_2=OH, R_3=H
HDCA: R_1=OH R_2=H , R_3=H
UDCA: R_1=H R_2=OH, R_3=H

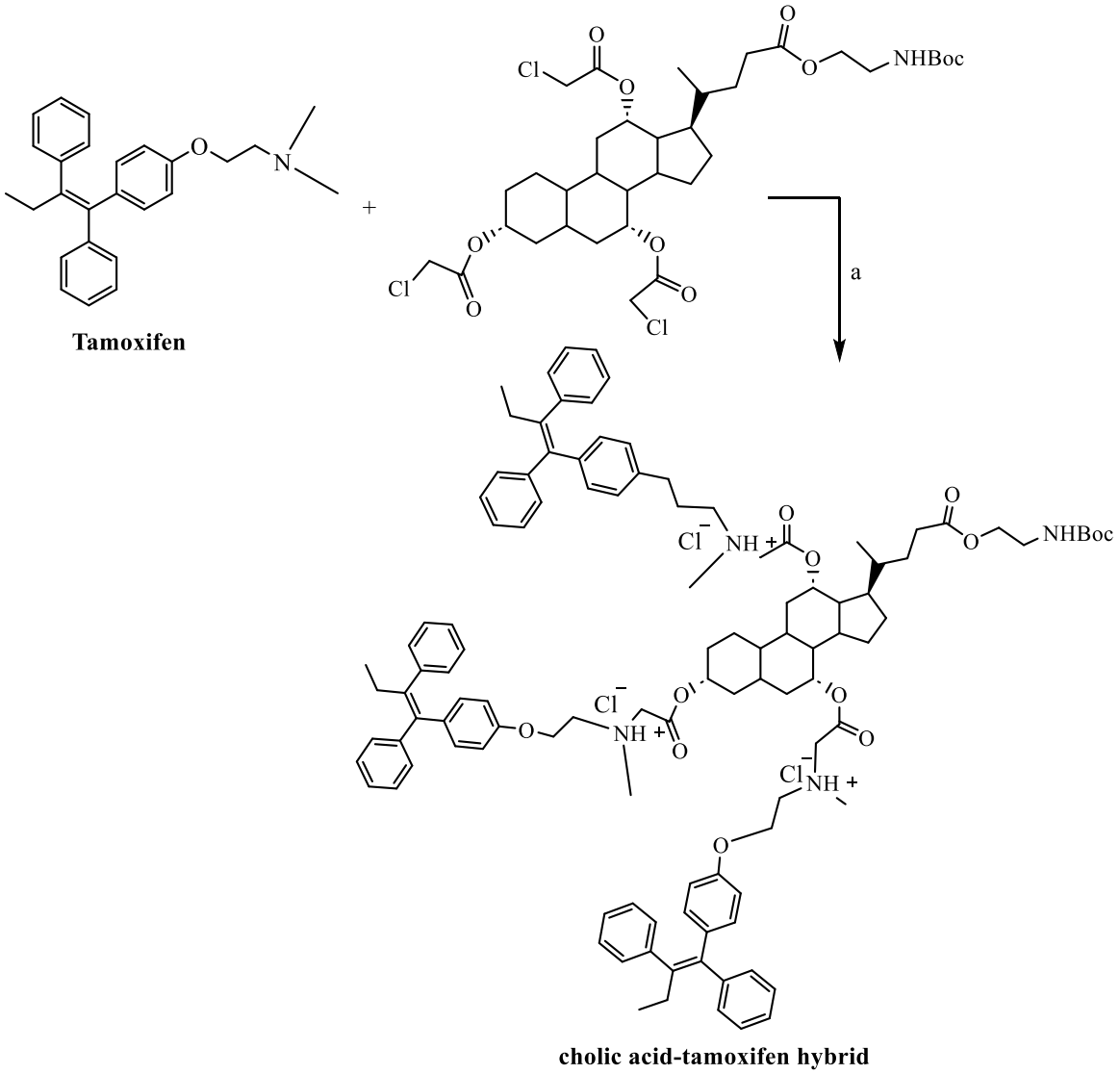
81: R_1=H R_2=OH, R_3=OH
82: R_1=H R_2=OH, R_3=H
83: R_1=OH R_2=H , R_3=H
84: R_1=H R_2=OH, R_3=H

Scheme-18: Synthesis of BA-Ara-C conjugates. Reaction conditions: Ara, C:BA:TEA:$C_lCOOCH_2(CH_3)_2$(1:1.2:1.5:1) -15°C; 30 mins; 78-87% yield.

These derivatives were evaluated against HL-60 cancer cells exhibited higher antitumor efficacy and enhanced drug availability via AOTP-mediated transport. Compound **81** demonstrated notable activity with an IC_{50} of 110.0 ± 13.7 nM. Additionally, pharmacokinetic studies in rats revealed that cytarabine attached with ursodeoxycholic acid doubled the oral bioavailability of cytarabine.

Cholic acid tamoxifen hybrids against breast cancer

Tamoxifen, a member of the nonsteroidal triphenylethylene derivative family [68], has been widely used for breast cancer treatment [69-72]. Sreekanth et al. [73] synthesized several bile acid tamoxifen hybrids with acid and amine head groups and evaluated their potential against ER^+ and ER^- breast cancer cells (Scheme 19). Among these, the derivative (CA linked to 3 TAM units) **85**, synthesized via a condensation reaction between a TAM conjugate and a cholic acid conjugate, exhibited the highest anticancer activity. Comparing this compound to tamoxifen alone, the activity of three of the four examined cancer cell lines increased by at least twofold (Table 9).



Scheme-19: Synthesis of cholic acid-tamoxifen hybrid. Reaction conditions: (a) Dichloromethane, trifluoroacetic acid, 6h, room tem.

The anticancer properties of the hybrid were evaluated in vivo using the murine 4T1 breast cancer model in Balb/c mice. Notably, the derivative demonstrated superior efficacy compared to tamoxifen (TAM) alone, reducing tumor volume by approximately 50% with a single dose, whereas TAM showed no significant effect at the same dosage.

Table-9: IC_{50} values of CA-TAM derivative.

Compound	IC_{50}μM				Ref.
	4T1	MCF-7	T47D	MDA-MB 231	[73]
TAM	12.25 ± 1.92	18.25 ± 4.19	22.41 ± 2.15	19.41 ± 4.47	
CA-TAM	5.28 ± 4.32	8.1 ± 3.82	9.42 ± 4.21	17.55 ± 5.85	

Antileukemic activity of cholic acid artemisinin hybrids against multidrug resistant cells

Letis et al. [74] designed several novel hybrids incorporating artemisinin and cholic acid moieties (Scheme 20). They evaluated the antileukemic properties of these hybrids against multidrug-resistant CEM/ADR5000 cells and drug-sensitive CCRF-CEM cells. Initially, cholic acid derivatives were synthesized and subsequently reacted with artemisinin derivatives via either ester or amide coupling to yield the desired hybrids. The novel hybrids exhibited IC_{50} values ranging from 0.345 mM to 7.159 mM against CEM/ADR5000 cells and from 0.019 mM to 0.192 mM against CCRF-CEM cells. Notably, the amide hybrid **86** was the most effective, showing IC_{50} values of 0.019 ± 0.001 mM in CCRF-CEM cells and 0.345 ± 0.031 mM in CEM/ADR5000 cells. In terms of cytotoxicity against multidrug-resistant CEM/ADR5000 cells, hybrid **86** outperformed doxorubicin (IC_{50} = 1.61 μM) [74].

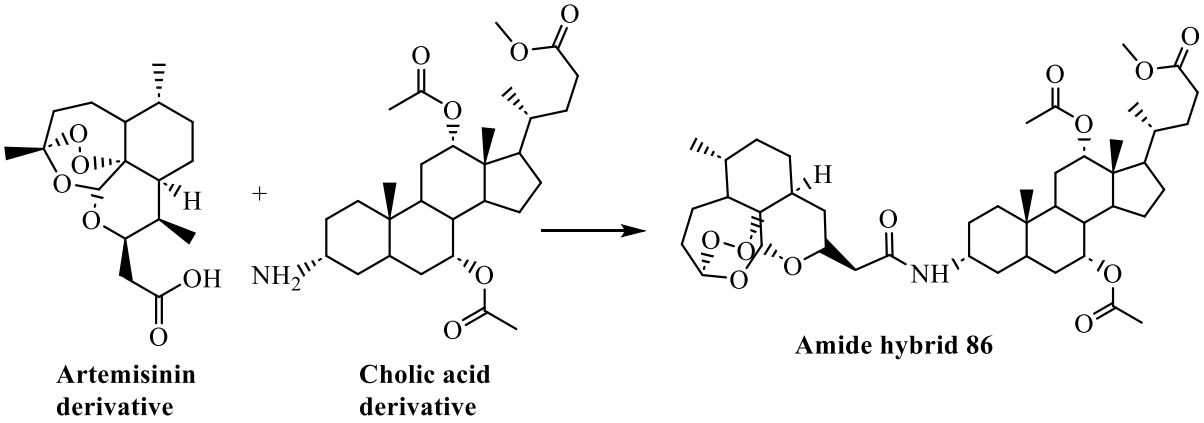
Cholic acid amino-substituted α-Cyanostilbene derivatives

α-Cyanostilbenes are often regarded as analogues of resveratrol, exhibiting potent anticancer properties across various cancer cell lines [75]. These compounds have also been found to possess antimicrobial activity [46]. Agarwal et al. evaluated the antitumor efficacy of a number of amino-substituted α-cyanostilbene derivatives—coupled with cholic acid (CA) and deoxycholic acid (DCA) amides—using the human osteosarcoma (HOS) cells (Scheme 21). A Knoevenagel condensation between 2-(4-nitrophenyl) acetonitrile and different aldehydes in the presence of catalytic amounts of piperidine marked the beginning of the production of these hybrid molecules. The intended α-cyanostilbenes were obtained by reducing the NO_2 group with overall yields of 80–85%. Finally, cholic acid (or deoxycholic acid) was coupled with the amino-substituted α-cyanostilbenes, affording the synthesis of desired compounds (**87–95**) in yields ranging from 52% to 88% [76].

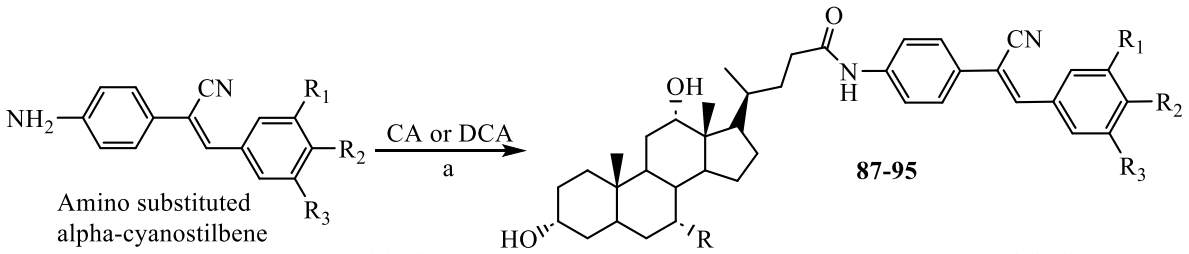
All the compounds expressed IC_{50} values ranging from 2 to 13 μM against HOS cells, with compound **93** being the most potent (IC_{50} = 2 μM).

Bile acid aryl/heteroaryl hybrids against colon and breast cancer

In another study, bile acid-based aryl/heteroaryl hybrids connected via amino acids were synthesized, and their cytotoxicity was evaluated [77].



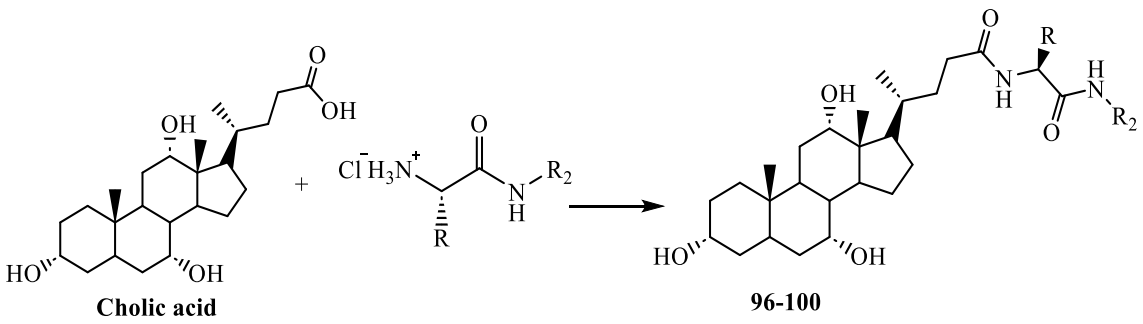
Scheme-20: Reaction conditions: EDCI.HCl (Ethyl (dimethylaminopropyl)carbodiimide hydrochloride, DMAP, DCM (Dichloromethane), 24h, 86% yield.



	HOS IC_{50}µM
87: R=OH, R_1=R_2=R_3=H	6.0
88: R=H, R_1=R_2=R_3=H	4.0
89: R=OH, R_1=R_3=H, R_2=OCH_3	8.0
90: R=OH, R_1=R_2=OCH_3, R_3=H	5.0
91: R=OH, R_1=R_2=R_3=OCH_3	13.0

	HOS IC_{50}µM
92: R=OH, R_1=R_3=H, R_2=CH_3	7.0
93: R=OH, R_1=R_3=H, R_2=OH	2.0
94: R=H, R_1=R_3=H, R_2=OH	20.0
95:R=OH, R_1=R_2=OH, R_3=H	7.0

Scheme-21: Synthesis of α-cyanostilbene hybrids. Reaction condictions: (a) EDC.HCl/HOBt/Et_3N, Dimethylformamide, 80°C, 1 h (52–88% yields).



Cholic acid

96: R=$CH(CH_3)CH_2CH_3$,	R_2=	MDA-MB-231 GI_{50}= 1.35µM
97: R=R=$CH(CH_3)CH_2CH_3$	R_2=	MDA-MB-231 GI_{50}= 1.41µM
98:R=CH_3	R_2=	U87MG GI_{50}= 2.49µM
99: R=$CH(CH_3)_2$	R_2=	U87MG GI_{50}= 2.46µM
100: R=CH_3	R_2=	MDA-MB-231 GI_{50}=4.52µM U87MG GI_{50}= 1.62µM

Scheme-22: Synthesis of aryl/heteroaryl hybrids. Reaction conditions: EDC·HCl/HOBt/DMF (Ethyl (dimethylaminopropyl) carbodiimide hydrochloride/ 1-hydroxybenzotriazole/ Dimethyl formamide), rt. 6–8 h.

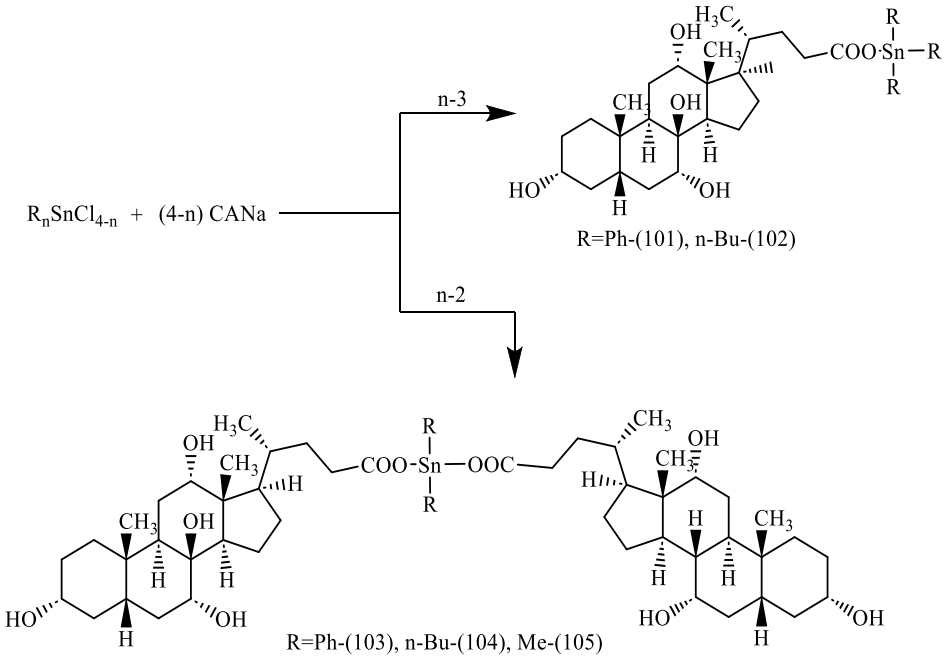
In the synthetic approach, L-aminoacyl aromatic and heteroaromatic amides were coupled with cholic acid (Scheme 22). The cytotoxicity of each conjugate was evaluated on a normal cell line (HEK293T), and their antiproliferative activity was assessed against three distinct human cancer cell lines representing colon (HT-29), breast (MDA-MB-231), and glioblastoma (U87) malignancies. Several CA analogues (compounds **96**, **97**, and **100**) showed promising potential against the breast cancer cell line, with GI_{50} values of 1.35, 1.41, and 4.52 μM, respectively—comparable to doxorubicin (1 μM) and significantly better than cisplatin (7.21 μM). In contrast, compounds **98**, **99**, and **100** demonstrated enhanced activity against the glioblastoma cell line, with GI_{50} of 2.49, 2.46, and 1.62 μM, respectively, outperforming both cisplatin (3.78 μM) and doxorubicin (2.60 μM) [78, 79].

Organotin (IV) derivatives of cholic acid against breast cancer

The anticancer activity of organotin compounds is well documented [80, 81]. These compounds disrupt mitochondrial function and damage cellular macromolecules, and collaborate with cell membranes to trigger apoptosis [80]. Recently, organotin (IV) derivatives of thiomide have shown anticancer activity against MCF-7 cells, although not all breast cancer cells responded similarly [82]. In light of this, Stathopoulou et al. developed organotin (IV) derivatives of cholic acid (Fig 9) and evaluated their anticancer potential against human breast adenocarcinoma cell lines, MCF-7 (hormone receptor-positive) and MDA-MB-231 (hormone receptor-negative).

Derivatives **101** and **102** were produced by reaction of sodium salt of cholic acid (CANa) with R_3SnCl, while derivatives **103**, **104**, and **105** were prepared using R_2SnCl_2 in a water/methanol solution [22] (Scheme 23). The in vitro bioactivity of compounds **101–105** was assessed using the SRB assay on MCF-7 and MDA-MB-231 cells. According to table 10, compound **105** exhibited no activity at doses as high as 30 μM. Moreover, compared to the di-organotin derivatives (**103–104**), the triorganotin derivatives (**101–102**) demonstrated superior activity against both MCF-7 and MDA-MB-231 cells. Overall, both triorganotin and di-organotin derivatives were 40–170 times more active than the clinically used cisplatin against these breast cancer cell lines.

TPI values greater than one indicates that an agent is more selective for malignant cells than for healthy ones, with higher scores reflecting a superior therapeutic profile. Accordingly, compound **102** exhibits a more favorable antitumor-to-toxicity ratio in MCF-7 cells (TPI = 1.30), while compound **103** shows enhanced selectivity against MDA-MB-231 cells (TPI = 1.11).



Scheme-23: Synthesis of compounds **101-105.**

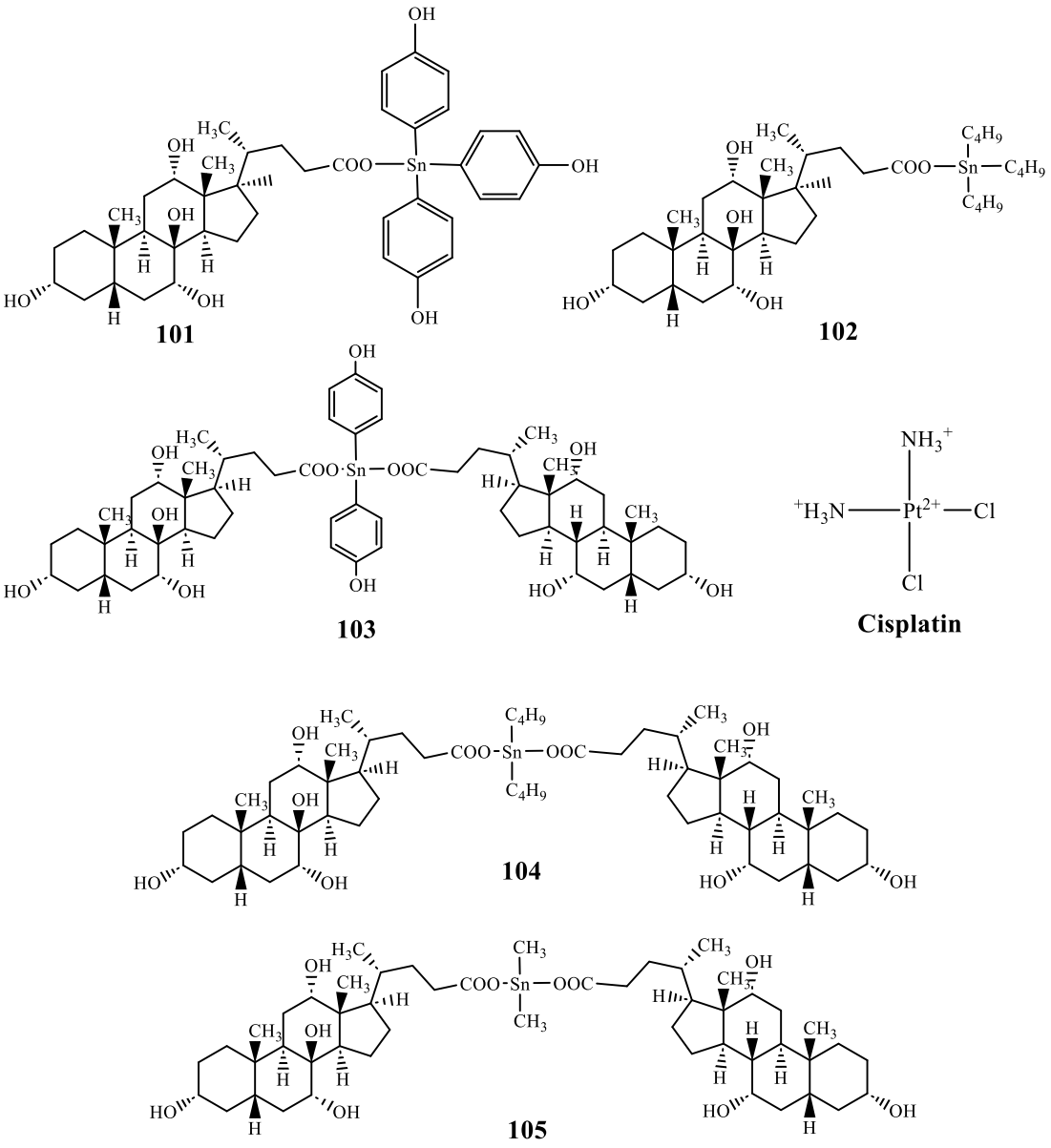


Fig. 9: Structures of organotin derivatives of cholic acid and cisplatin.

Table-10: IC_{50} values of compounds 101-105.

Compounds	IC_{50}(μM)			TPI		Ref.
	MCF-7	MDA-MB-231	MRC-5	MCF-7	MDA-MB-231	[83]
101	**0.10 ± 0.00**	**0.16 ± 0.01**	**0.07 ± 0.00**	**0.70**	**0.44**	
102	**0.13 ± 0.00**	**0.35 ± 0.01**	**0.17 ± 0.00**	**1.30**	**0.49**	
103	**1.32 ± 0.03**	**0.68 ± 0.03**	**0.76 ± 0.02**	**0.58**	**1.11**	
104	**0.42 ± 0.01**	**0.40 ± 0.02**	**0.39 ± 0.01**	**0.93**	**0.98**	
105	**>30**	**>30**	**>30**			
Cholic acid	**>30**	**>30**	**>30**			
Cisplatin	**5.5 ± 0.4**	**26.7 ± 1.1**	**1.1 ± 0.2**	**0.20**	**0.04**	

Bile acid derivatives against colon cancer

Numerous bile acid (BA) receptors are present on the cell membranes of colon tissues. BAs with their hydrophilic and hydrophobic features, facilitate targeted drug delivery to the colon [84]. In addition, BAs possess inherent anti-inflammatory [85] and anti-cancer properties. To combat colon cancer, Wang et al. synthesized several BA derivatives and evaluated their anticancer efficacy. The cholic acid derivatives were numbered **106–113**, while other derivatives were prepared from different bile acids [86]. Specifically, derivative **106** was synthesized by reacting cholic acid with 2-hydroxybenzamide; derivatives **107–112** were obtained through esterification; and derivative **113** was produced by conjugating methionine with cholic acid (Scheme 24).

Among these compounds, four cholic acid derivatives (**109**, **110**, **111**, and **112**) demonstrated superior activity against HCT116 cells compared to the other compounds and the positive control 5-fluorouracil (5-FU). Their IC_{50} values ranged from 21.32 μmol L^{-1} to 28.90 μmol L^{-1}. Furthermore, these derivatives suppressed both colony formation and the migration as well as invasion of HCT116 cells. They also triggered apoptosis, caused G2/M phase cell cycle arrest, disrupted the function of mitochondria, elevated ROS level, and downregulated Bcl-2 and p-STAT3 in HCT116 cells (Fig 10) [86].

Cholic acid conjugated oxaliplatin against liver cancer

Only a few numbers of chemotherapy drugs, including cisplatin and oxaliplatin, show effectiveness against liver cancer [87]. However, oxaliplatin is associated with severe toxicity due to its poor specificity [88], necessitating the development of prodrugs that can be activated at specific target sites. To address this need, Jiand et al. developed LLC-202, a cholic acid-conjugated oxaliplatin, as a novel prodrug for liver cancer [89]. Similarly, Lan et al. synthesized LLC-202 **(126)**, a cholic acid-conjugated carboplatin, aimed at targeting the liver. In Jiand et al.'s study, 3-amino-2-cyclobutane-1,1-dicarboxylate was used as a connector between the oxaliplatin analogue and cholic acid, with the cholic acid being firmly attached to the connector through –CONH bond (Fig 11).

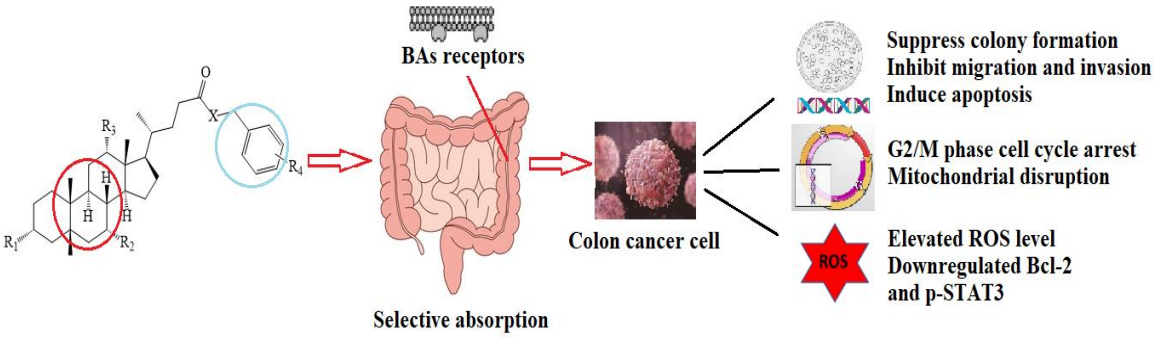
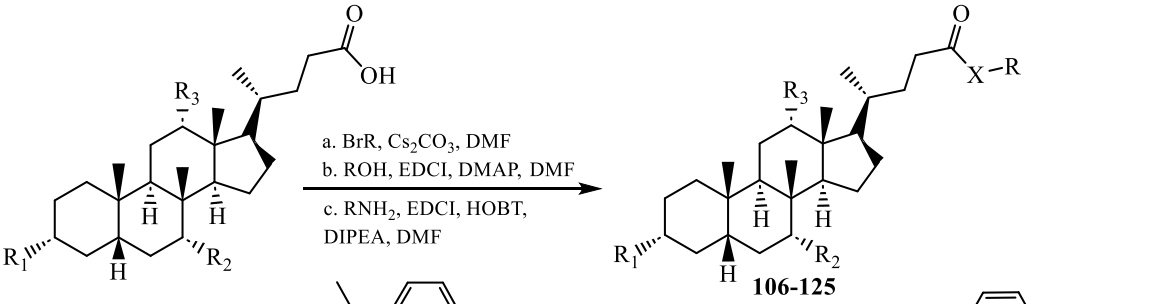


Fig. 10: Anticancer mechanism of action of cholic acid derivatives.



106: R_1=R_2=R_3=OH, X=NH, R=

107: R_1=R_2=R_3=OH, X=O, R=

108: R_1=R_2=R_3=OH, X=O, R=

109: R_1=R_2=R_3=OH, X=O, R=

110: R_1=R_2=R_3=OH, X=O, R=

111: R_1=R_2=R_3=OH, X=O, R=

112: R_1=R_2=R_3=OH, X=O, R=

113: R_1=R_2=R_3=OH, X=NH, R=

114: R_1=R_2=OH,R_3=H X=O, R=

115: R_1=R_2=OH,R_3=H X=O, R=

116: R_1=R_2=OH,R_3=H X=O, R=

117: R_1=R_2=OH,R_3=H X=O, R=

118: R_1=R_2=OH,R_3=H X=O, R=

119: R_1=R_2=OH,R_3=H X=O, R=

120: R_1=R_2=OH,R_3=H X=O, R=

121: R_1=R_2=OH,R_3=H X=O, R=

122: R_1=OH, R_2=R_3=H X=O, R=

123: R_1=OH, R_2=R_3=H X=O, R=

124: R_1=R_2=ßOH,R_3=H X=O, R=

125: R_1=R_2=ßOH,R_3=H X=O, R=

Scheme-24: Synthesis of bile acid derivatives **(106-125)**.

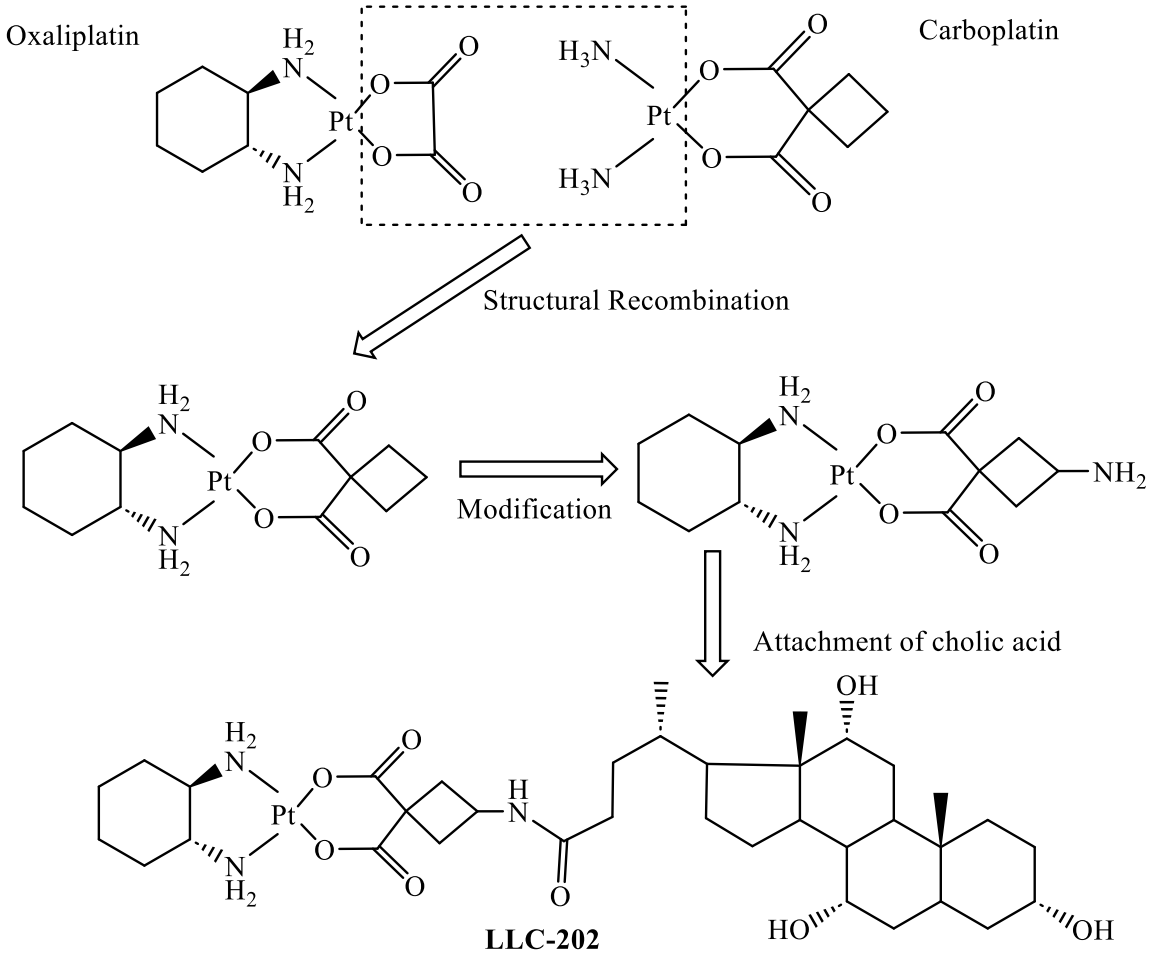
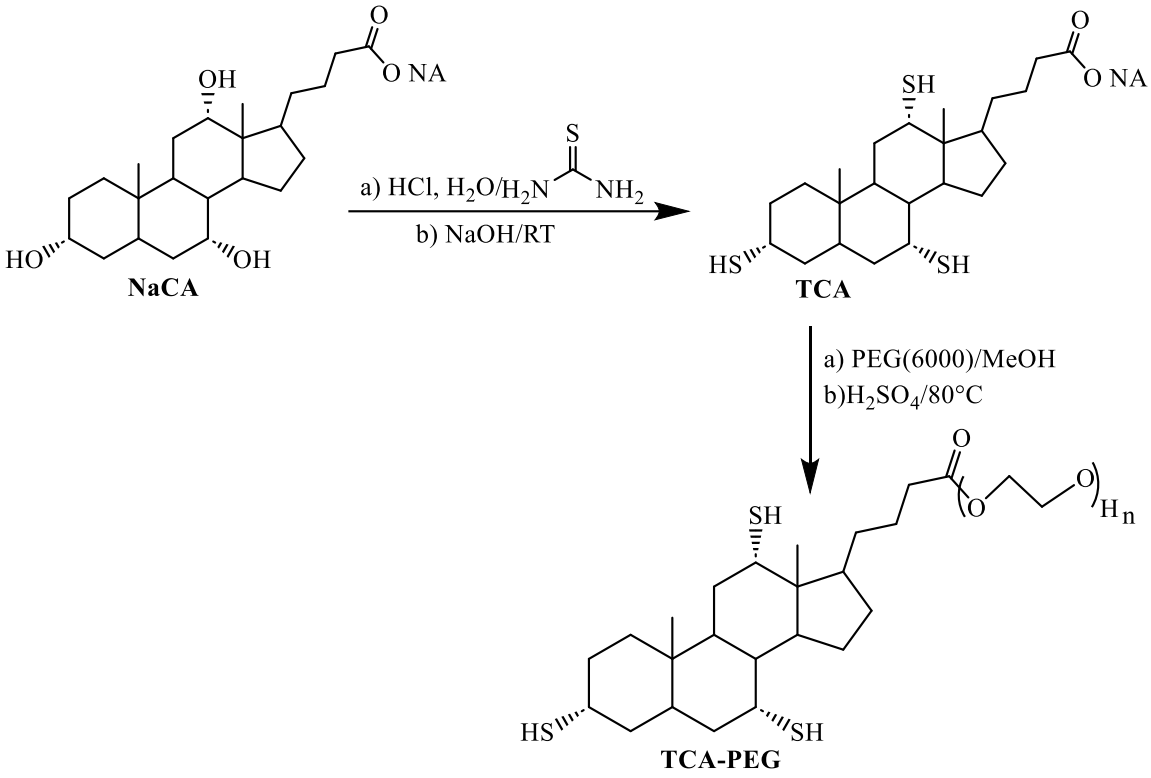


Fig 11: Synthetic approach for LLC-202, an oxaliplatin prodrug. It is composed of a combination of carboplatin and oxaliplatin, with cholic acid serving as the liver-targeting moiety and 1, 1-cyclobutane-dicarboxylic acid as the linker.

Following intravenous administration in Sprague-Dawley rats, pharmacokinetic studies revealed that LLC-202 is predominantly transported to and deposited in the liver. Compared to oxaliplatin, human liver cancer cells absorb LLC-202 more readily than healthy human liver cells. In C57BL/6 mice with primary hepatocellular carcinoma, LLC-202 demonstrated superior in vitro anticancer activity and efficacy relative to oxaliplatin. Moreover, LLC-202 significantly prolonged the survival of tumor-bearing animals by inducing apoptosis and inhibiting cancer cell proliferation, while exhibiting lower cytotoxicity toward healthy human liver cells [90]. Baskaran et al. developed thiomeric micelles composed of cholic acid (CA) with enhanced mucoadhesive properties by functionalizing thiol groups and conjugating polyethylene glycol (PEG). The synthesis began with sodium cholate, which was converted into thiocholic acid. Subsequent pegylation produced pegylated thiocholic acid (TCA-PEG), and this was then used to encapsulate gold and silver nanoparticles [91] (Scheme 25).



Scheme 25: Synthesis of polyethylene glycol conjugated thiocholic acid (TCA-PEG) **(127)**.

The cytotoxicity and apoptotic effects of TCA-PEG polymeric micelles encapsulating silver (AgNPs) and gold (AuNPs) nanoparticles were evaluated in MCF-7 breast cancer cells utilizing MTT assay. Cells were treated with varying concentrations of AgNPs and AuNPs for 24 and 48 hours. The IC_{50} values were calculated to be 50 µg/mL for AgNPs and 60 µg/mL for AuNPs, suggesting that AgNPs are more potent at lower concentrations. Reaching highest tested concentration of 80 µg/mL after 48 hours, AuNPs induced a greater level of cell death (34%) compared to AgNPs (20%) [91].

Targeted drug delivery applications

Antimicrobial applications

Emerging research identifies bile acids (BAs) as powerful tools for improving the safety and delivery of hydrophobic drugs. Because BAs like cholic and deoxycholic acid (DCA) have a unique amphiphilic structure, they can naturally form micelles and navigate biological pathways, effectively acting as "Trojan horse" carriers [92]. Specifically, Cholic acid (CA) derivatives are increasingly recognized as viable therapeutic alternatives, largely due to their established antiproliferative and antimicrobial profiles. Among recent developments, ceragenines have emerged as a novel class of compounds engineered to structurally emulate the cationic, facially amphiphilic architecture of endogenous antimicrobial peptides (AMPs). Comparative analyses indicate that ceragenines replicate the antibacterial behavior of these natural peptides [93].

In the context of drug delivery system, Faustino et al., developed mixed micelles by pairing DCA with biocompatible dimeric anionic lipoamino acids. In this system, the hydrophobic side of the DCA molecule buries itself deep within the micellar core, forcing the structure to lose its standard spherical shape and become asymmetrical. This structural distortion stabilizes Amphotericin B (AmB) as individual monomers, preventing the dangerous aggregation that typically causes kidney damage and hemolysis. Consequently, this bile acid-based approach maintains the drug's ability to destroy *Candida albicans* while significantly lowering its toxicity to human cells [94].

Tang's research group recently synthesized a library of macromolecular antimicrobial amphiphiles derived from cholic acid (CA), lithocholic acid, and deoxycholic acid. Their objective was to elucidate the

impact of facial amphiphilicity on bacterial membrane disruption by varying spacer chain lengths and molecular weights. When evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, the study revealed that lower molecular weight polymers derived from CA exhibited superior performance compared to both their higher molecular weight counterparts and other bile acid analogues. Notably, these polymers demonstrated more pronounced efficacy against Gram-negative bacteria than Gram-positive strains [95].

Anticancer applications

Most research on drug delivery has focused on polymeric devices for cancer treatment [96], but cholic acid (CA) has recently emerged as a valuable material for creating site-specific therapies [97]. Due to its unique properties, CA has been used to develop macromolecular micelles that show great promise for targeted cancer care [98]. For instance, Zhu and colleagues designed CA-conjugated hydrophobic polymers capable of carrying the chemotherapy drug doxorubicin (DOX). In their study, they analyzed three different formulations to determine how the drug was loaded and released. They discovered that these CA-based systems not only carried a high amount of the drug but also responded to changes in acidity. Specifically, the micelles released 50% of the drug at a pH of 5.0, compared to only 12% at a neutral pH of 7.4. Since tumor environments are typically more acidic than healthy tissue, this pH-responsive mechanism allows the drug to be released precisely where it is needed, proving that CA-based systems are effective vehicles for targeted drug delivery [99, 100].

Kim and his colleagues recently introduced a new approach to cancer chemotherapy using amphiphilic polymers based on cholic acid (CA). They developed specific carriers known as MCPEI-CAs by linking CA with methyl cellulose polyethylenimines (MCPEI). These nanocarriers proved effective at trapping the anticancer drug doxorubicin (DOX), achieving loading efficiencies of 58.0% and 23.2%. Furthermore, the team proposed that these drug-loaded nano-aggregates could effectively bypass the defenses of multidrug-resistant cancer cells, with their effectiveness increasing at higher concentrations [101].

Shao and his team developed a series of random and block copolymers using cholic acid (CA) and oligoethylene glycol through the ROMP synthesis method. These materials were amphiphilic, allowing them to self-assemble into micelles in water, and they demonstrated distinct responses to temperature changes. Specifically, as the temperature rose from 20 to 54 °C, the micelles made from block copolymers shrank, whereas the random copolymer micelles expanded. Both types were able to carry the cancer drug paclitaxel (PTX), but their release rates differed significantly: the block copolymers released approximately 78% of the drug, while the random copolymers released only 24% [102]. In a related area of research, Zhang and colleagues utilized CA-based polymers to target liver cancer. They engineered nanoparticles containing CA that could interact specifically with bile acid transporters on cell membranes. This interaction significantly enhanced the cells' ability to absorb the nanoparticles, making them a highly effective tool for delivering drugs directly to hepatoma cells [103].

The examples reviewed above highlight the extensive potential of Cholic Acid-Polymers (CAPs) within the drug delivery sector. It is highly likely that the near future will bring even more advanced and innovative CA-based materials designed to improve treatment efficiency.

Conclusion

In conclusion, the integration of cholic acid into therapeutic design has emerged as a promising strategy to address some of the most pressing challenges in modern medicine. The reviewed studies demonstrate that cholic acid-based conjugates and hybrids exhibit enhanced drug delivery, increased selectivity for malignant cells, and potent cytotoxic effects against different cancer cell lines. Notably, conjugation with agents such as cytarabine and tamoxifen has led to significant improvements in pharmacokinetic profiles and liver-targeting capabilities, thereby outperforming conventional treatments. In parallel, the development of cholic acid derivatives linked to platinum drugs, organotin compounds, and artemisinin analogues has provided compelling evidence of their ability to overcome drug resistance and reduce systemic toxicity. Furthermore, cholic acid’s unique amphiphilic nature has been effectively exploited in the creation of advanced delivery systems, including thiomeric micelles encapsulating metallic nanoparticles, which enhance mucoadhesion and facilitate targeted therapy. These innovative approaches not only broaden the therapeutic potential of cholic acid derivatives but also pave the way for the next generation of prodrugs and hybrid molecules with improved safety and efficacy. Despite these promising advances, challenges remain in optimizing synthetic routes, fully elucidating structure–activity relationships, and validating long-term safety in clinical settings. Future research should

focus on these areas to harness the full potential of cholic acid-based therapeutics. Overall, the versatility and efficacy of cholic acid derivatives position them as a powerful platform for the development of innovative treatments for cancer, infectious diseases, and beyond.

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