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CONVENIENT AND EFFICIENT SYNTHESIS OF SOME NOVEL CHOLIC ACID DERIVATIVE AND ITS ANTIMICROBIAL EVALUATION.

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Abstarct :- A convenient and competent synthesis of a series of eleven new Cholic acid derivative (A-K) is described starting from Cholic acid. The invitroantimicrobial estimation of the newly synthesized Cholic acid derivative was performed against four bacterial strains i.e.two Gram-positive bacteria viz. Bacillus subtilis(MTCC 441) & Staphylococcus epidermidis(MTCC 6880), two Gram-negative bacteria viz. Escherichiacoli(MTCC 1652) & Pseudomonas aeruginosa(MTCC 424) and twofungal strains viz. Candida albicans(MTCC 227) & Aspergillus niger(MTCC 8189) by sequential dilution technique. The derivatives 2e, 2f, 2gand 2kwere found to exhibit better antibacterial activity than other tested compounds against B. S.epidermidis.

Key words:-Cholic acid ,HATU(1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate),Antimicrobial

1. Introduction:-

Cholic acid, a main bile acid, is a biosurfactant involved in the absorption of dietetic lipids. With the carboxylate group as the ionic head group it resembles a ™traditional surfactant.However, the steroid part with three hydroxy groups on one side is often considered to be a facial amphiphile. These hydroxy groups can have specific connections, such as those found in enclosure compounds,[1, 2]organogelators,[3 -6] and receptors [7, 8] based on cholic acid. in addition, the hydroxygroups, each with a different reactivity,[9] make cholic acid an best root for a synthetic method. The polarization of the hydroxy groups can be improved to emphasize the facial amphiphilicity of the steroid unit. The resultant facial amphiphiles include lots of applications in ion transport,[10, 11] combinatorial chemistry,[12]vesicle fusion,[13]and development of membrane permeability.[14] Even facial amphiphiles with three ionic groups are reported.[15,16] However, the properties of these cationic (NH_3^+) or anionic (COO^-**)** facial amphiphiles are dependent on the pH of the solution. In this,a series of new facial amphiphiles with a stable ionic character is presented. Three cationic trimethylammoniumgroups were close to cholic acid and the carboxylate group was esterified, to yield a new class of three-headed surfactants. We know of only one other example of a surfactant with three permanent ionic head groups and only one hydrophobictail.[17]We report on the aggregation of this new type of surfactant into spherical micelles and on its antimicrobialactivity.

2. Materials and methods

All reagents were used as obtained from commercial suppliers not including additional purification. Melting points (mp, °C) of all the synthesized compounds were determined on an electrothermal apparatus in open capillaries and are uncorrected. The purity of synthesized compounds was checked using precoated TLC plates (Merck Keiselgel F_{254}) and visualization was attained via UV light. The FTIR spectra were recorded on IR affinity-1 FTIR (Shimadzu) spectrometer in KBr and wave numbers (ν_{max}) are reported in cm^{-1} ^{1}H NMR spectra were scanned on Bruker AVANCE II NMR spectrometer operating at 400 MHz using DMSO-d_6 as solvent and tetramethylsilane(TMS) as internal standard. Chemical shift (δ)values are expressed

in parts per million(ppm) and coupling constants (*J*) are reported in Hertz(Hz). Mass spectra were recorded on Waters Quadrupole Detector (TDQ).

3. Result and discussion

3.1 Chemistry

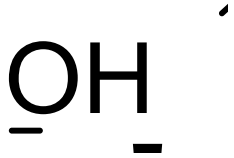
We have replaced the carboxylic acid moiety of cholic acid (1) with an amide functionality derived from various amine. On the basis of literature survey and references the compoundswas synthesis via Scheme-1. In Scheme-1 we use Acetic anhydride, Pyridine and oxlayl chloride which is hazardous and compound was syntheis via four step overall yield is also poor.so we optimized the method via using different acid amine catalyst and solvent which was mention in table-1.

Table-1

Sr. No	Name of reagent	Base	Solvent	Yield	Time in hr
1	HATU	DIPEA	DMF	**93%**	**0.5**
2	HATU	DIPEA	THF	87%	1
3	EDCI,HOBT	DIPEA	THF	60%	10
4	EDCI,HOBT	DIPEA	MDC	58%	10
5	DCC	TEA	THF	49%	14
6	DCC	TEA	THF	52%	14

3.2 Comparison of Scheme-1 with Scheme-2

Scheme-1



Scheme-2

3.3. General procedure for the synthesis of cholic acid derivative 2(a-k)

To a stirred solution of cholicacid DMF was added. cooled the reaction mass upto 0°to 5°C.Then added HATU (2.0 eq) and DIPEA (3.0 Eq) at 0° to 5°C. Stirred the reaction mass for 30 min at 0° to 5° C. Then added Amine (1.2 eq) stirred the reaction mass at Room temp for 45-60 min. The reaction mass was quenched in cold water and extracted with ethyl acetate.The reaction mass was purified by column chromatography. The product was eluted out at 10% methanol in dichloromethane. The compound was triturated in pentane.

3.4. Experimental

3.4.1 (R)-N-(3,4-difluorophenyl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2a)

This cholic acid derivative was synthesized as per to the general procedure. Yield 93%, as white solid, m.p. 160–162°C,^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 7.21–7.30 (m,skeletal 2H CH-aromatic); 7.69 (s, 1H, CH Aromatic); 10.12 (s,1H, CONH).

3.4.2 (R)-N-(4-chlorophenyl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2b)

This cholic acid derivative was synthesized as per to the general procedure. Yield 81%, as Brown solid, m.p. 182-183°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.56 (S, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 4.00-4.10(2H d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 7.29–7.31 (m,skeletal 2H CH-aromatic); 7.69 (s, 1H, CH Aromatic); 9.17 (s,1H, CONH).

3.4.3 (R)-N-(pyridin-2-yl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2c)

This cholic acid derivative was synthesized as per to the general procedure.Yield 71%, as yellow solid, m.p. 189–190°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 7.29 (1H ,T ,CH-aromatic); 7.99 (1H,T,CH-aromatic); 8.09 (1H ,d,CH-aromatic); 8.40-8.42 (d, 1H, N-CH Aromatic); 10.12 (s,1H, CONH).

3.4.4 (R)-N-(2,5-dimethoxyphenyl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2d)

This cholic acid derivative was synthesized as per to the general procedure. Yield 75%, as Grey solid, m.p. 178–179°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 6.70 (1H,d,CH Aromatic); 7.10 (1H,d,CH Aromatic); 7.69 (s, 1H, CH Aromatic); 10.12 (s,1H, CONH).

3.4.5 (R)-N-(pyrazin-2-yl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2e)

This cholic acid derivative was synthesized as per to the general procedure. Yield 66%, as Brownish solid, m.p. 244-246°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 8.35 (1H,d,CH Aromatic); 8.48 (1H,d,CH Aromatic); 8.62 (s, 1H, CH Aromatic); 10.15 (s,1H, CONH).

3.4.6 (R)-N-(pyrimidin-5-yl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2f)

This cholic acid derivative was synthesized as per to the general procedure. Yield 62%, as cream solid, m.p. 169–171°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 9.10 (1H,S,CH Aromatic); 9.21 (1H,d,CH Aromatic); 9.21 (s, 1H, CH Aromatic); 10.15 (s,1H, CONH).

3.4.7 (R)-N-(pyrimidin-4-yl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2g)

This cholic acid derivative was synthesized as per to the general procedure. Yield 65%, as yellow solid, m.p. 164-166°C, .1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 8.02 (1H,D,CH Aromatic); 8.82 (1H,S,CH Aromatic); 8.92 (s, 1H, CH Aromatic); 10.09 (s,1H, CONH).

3.4.8 (R)-N-(3-chloro-4-fluorophenyl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2h)

This cholic acid derivative was synthesized as per to the general procedure. Yield 62%, as white solid, m.p.208-209°C,1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 8.02 (1H,D,CH Aromatic); 8.82 (1H,S,CH Aromatic); 8.92 (s, 1H, CH Aromatic); 10.09 (s,1H, CONH).

3.4.9 (R)-N-(pyridin-3-yl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2i)

This cholic acid derivative was synthesized as per to the general procedure. Yield 58%, as Cream solid, m.p. 182-185°C,1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 7.38 (1H,T,CH Aromatic); 8.29 (1H,D,CH Aromatic); 9.12 (s, 1H, CH Aromatic); 9.36 (1H,S, CH Aromatic); 10.09 (s,1H, CONH).

3.4.10 (R)-N-(4-fluoro-3-methylphenyl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2j)

This cholic acid derivative was synthesized as per to the general procedure. Yield 45%, as Cream solid, m.p. 192–193°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH3); 2.34 (S, Aromatic-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 7.10 (1H,D,CH Aromatic); 7.41(1H,D,CH Aromatic); 7.75 (s, 1H, CH Aromatic); 10.09 (s,1H, CONH).

3.4.11 (R)-N-(pyrimidin-2-yl)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamide (2k)

This cholic acid derivative was synthesized as per to the general procedure. Yield 49%, as Orange solid, m.p. 189–172°C,.1 H NMR (300 MHz, DMSO-d_6, in ppm): 0.59 (s, 3H, 18-CH_3); 0.78 (s, 3H, 19-CH_3); 0.95 (d, 3H, J=6, 21-CH_3); 2.34 (S, Aromatic-CH_3); 1.14–2.39 (m, ca 24H, skeletal $CH_2$and CH), 3.16 (bs, 1H, H-3b); 3.59 (bs, 1H, H-7b); 3.76 (bs, 1H, H-12b); 3.83(6H,S-Ome) 4.00-4.10(d, 1H,J=3.17, 3-OH,1H, 7-OH); 4.30 (d, 1H,J=3.76, 12-OH); 7.49 (1H,T,CH Aromatic); 8.81(2H,S,CH Aromatic);10.09 (s,1H, CONH)

3.5 Biology

Ampicillin, Norfloxacin, Chloramphenicol and Ciprofloxacin were used as Standard controls. Solution of different concentrations of Ampicillin, Norfloxacin, Chloramphenicol and Ciprofloxacin and Compounds 2(a–k) were prepared by dissolving them in DMSO. Eleven serial dilutions prepared by halving the concentration of the stock solution with initial concentration of (250 mg/ml). Microorganism's suspensions at 105 CFU (colony forming units)/ml wereinoculated in the wells. The plates were incubated at 37°C for 24 h.The minimum inhibitory concentration (MIC) values were determined according to turbidity test.[18]

In summary, we have synthesized a new series of cholic acid derivatives and evaluated for their anti microbial and anti becterial theraputic activity. Among them molecules 2e, 2f, 2g and 2k exhibited promising activity against Ampicillin, Norfloxacin, Chloramphenicol and Ciprofloxacin and molecule 2c and 2i showed significant activity at higher concentration which are mention in

Table-2. However, this structure activity relationship (SAR) might give these compounds a potential suitable tool for designing new molecules having specific selectivity for anti microbial and anti becterial theraputic activity. In addition, the cholic acid moiety can be optimized as a lead molecule for anti microbial and anti becterial theraputic activity.

Table 2
Minimum inhibitory concentration (MIC) in mg/ml of compounds 2A-k

Table 2
Minimum inhibitory concentration (MIC) in mg/ml of compounds 2A-k

Compound	Ar	Gram positive bacteria			Gram negative bacteria			Fungi	
		S.P. MTCC	B.S. MTCC	C.T. MTCC	E.C. MTCC	S.T. MTCC	V.C. MTCC	C.A. MTCC	A.F. MTCC
		###	441	449	443	98	###	227	###
2a	3,4-difluoroaniline	500	500	500	500	500	500	###	500
2b	4-chloro aniline	200	500	**200**	250	500	**63**	###	**100**
2c	pyridin-2-amine	**100**	**150**	**100**	200	**100**	200	**100**	250
2d	2,5-dimethoxyaniline	500	**100**	500	**100**	250	**100**	**500**	500
2e	2-Amino pyrazine	**80**	**100**	**50**	100	**100**	150	**200**	**100**
2f	5-Amino pyrimidine	200	**75**	**100**	**90**	100	**100**	100	90
2g	4-Amino pyrimidine	**100**	250	50	100	**63**	90	**100**	250
2h	3-chloro-4-fluoroaniline	250	**100**	**100**	200	250	**100**	###	**100**
2i	3-amino pyridine	125	**250**	500	200	**100**	250	**250**	**100**
2j	4-fluoro-3-methylaniline	**100**	500	**100**	**63**	200	250	**250**	500
2k	pyrimidin-2-amine	125	**100**	**63**	200	**100**	**63**	**100**	###
Ampicillin		100	250	250	100	100	100	n. t.[a]	n. t.
Norfloxacin		10	100	50	10	10	10	n. t.	n. t.
Chloramphenicol		50	50	50	50	50	50	n. t.	n. t.
Ciprofloxacin		25	50	100	25	25	25	n. t.	n. t.

4. Conclusion

In summary, we have explored the utilization of Cholic acid derivatives as possible lead compounds in opposition to some Grampositive and Gram-negative species. This in crack, opens up the opportunity of elaborating on these derivatives for innovation more active broad spectrum antimicrobial derivatives of cholic acid.

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