

STUDY OF ANTIMICROBIAL ACTIVITY OF 4-METHYL-7-OXYCOUMARIN DERIVATIVES

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ABSTRACT:-

All the antibacterial drugs we use today were discovered between 1940s and 1970s through empirical screening program to identify inhibitors on the basis of their ability to prevent bacterial growth. Though development of these drugs caused impressive global reduction in bacterial infections but there prolong, excessive and indiscriminate use has contributed to the emergence of multi-drug resistance bacteria. A series of 7-oxycoumarinyl amino alcohols were designed and synthesized for the first time via an easy, convenient and efficient route. The antimicrobial results showed that some of the compounds showed moderate to excellent in vitro activity against different Gram-positive and Gram-negative bacterial strains and fungi with MIC values between 1.09 and 75 µg/mL.

Keyword: - antibacterial, Drugs, Alcohols, compounds, antimicrobial.

INTRODUCTION:-

The increasing prevalence of disease causing bacteria and fungi that are resistant to current drug therapy represents an alarming threat to public health. The problem has further aggravated with the association of bacterial and fungal infections with other diseases mainly in immunocompromised persons. Despite the major cause of morbidity and mortality these infections are given low priority and the pipeline for the discovery of new antimicrobial agents has decreased. All the antibacterial drugs we use today were discovered between 1940s and 1970s through empirical screening program to identify inhibitors on the basis of their ability to prevent bacterial growth. Though development of these drugs caused impressive global reduction in bacterial infections but there prolong, excessive and indiscriminate use has contributed to the emergence of multi-drug resistance bacteria. Some of these resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* (MRSE), vancomycin resistant *Enterococcus faecium* (VRE) and carbapenems-resistant *Enterobacteriaceae* are capable of surviving the effect of most of the currently used antibiotics. Similarly, emerging resistance to

the existing antifungal drugs such as amphotericin-B, azoles and echinocandins etc is also a major cause of concern in the antifungal therapy. Thus, the situation is warranting discovery of alternative drugs involving new molecules with broader spectrum and fewer side effects that are capable of treating resistant microbial strain.

The structural resemblance of coumarin compounds, containing 1, 2-benzopyrone skeleton, with clinical anti-infective quinolone drugs, containing enzopyridone backbone, provides ample opportunities to develop new antimicrobial agents. Some naturally occurring compounds novobiocin, chlorobiocin and coumermycin A₁, isolated from *Streptomyces* species, are aminocoumarin class of antibiotics that target DNA gyrase. However their complex synthesis, side effects, relatively less potency against Gram-negative bacteria and poor water solubility prevent them from being clinically successful drugs.

Due to encouraging antimicrobial potential numerous efforts have been made not only towards the isolation and purification of naturally occurring coumarins from variety of plants and animals but also towards the development of synthetic coumarin compounds with antimicrobial properties. Several coumarin derivatives were also tested for their antifungal activity although no effective coumarins as clinical antifungal drug have been developed until now.

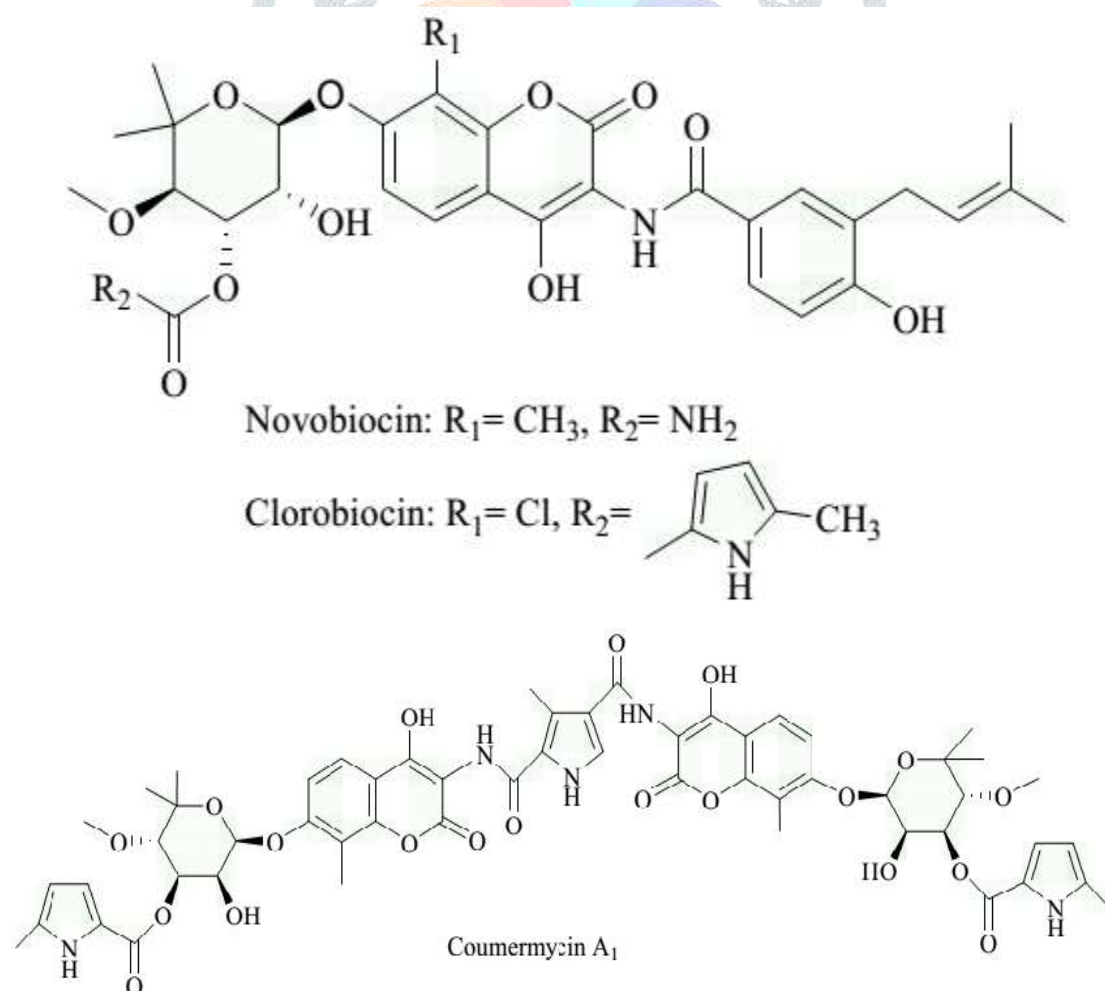


Figure 1: Structures of novobiocin, clorobiocin and coumermycin A₁

Several β -amino alcohol derivatives have been examined for their antimicrobial activity and some of them are used as active pharmaceutical constituents. Ethambutol, a bis-(β -amino alcohol), is a bacteriostatic antimycobacterial drug that targets the arabinosyl transferase enzyme involved in the biosynthesis of arabinan in mycobacterial cell wall. From the structure of ethambutol, a modern chemical approach was undertaken leading to the synthesis of many chemical libraries of diaminated analogues. Recently, based on the hit-compound 3-[3'-(4''-cyclopent-2'''-en-1'''-ylphenoxy)-2'-hydroxypropyl]-5,5 dimethylimidazolidine-2,4-dione, synthesis of some new hydantoin based β -amino alcohols as specific inhibitors of mycobacterial N-acetyltransferase (NAT) enzymes has been reported. Some chiral N-alkylated β -amino alcohols synthesized from phenylpropanolamines also showed good antimicrobial activity. In addition, literature survey revealed that a number of compounds containing β -amino alcohol moiety have been designed, synthesized and evaluated for their antimicrobial activity involving several strains. A variety of glycosylated amino alcohols are also reported for their good antimycobacterial activity. However, antimicrobial activity of coumarins bearing amino alcohol side chains has not been reported so far. Based on these findings, we have planned to synthesize a new class of compounds by attaching β -amino alcohol moiety to the coumarin skeleton and to investigate the antimicrobial activity of hybrid molecules. It is well documented that bioactivity of coumarin scaffold depends largely on the substitution pattern in aromatic and pyrone ring. Furthermore, number of structure activity relationship (SAR) studies on coumarin derivatives have illustrated that substitute at 7-position and methyl group at 4-position are important for antimicrobial activities. Therefore, we have chosen 7-hydroxy-4-methylcoumarin as starting material in the present study. Keeping in mind the fact that lipophilicity of the compound play crucial role in eliciting its biological response, substituents in amino alcohol moiety in the target compounds were systematically investigated for optimum pharmacophoric requirement for antimicrobial activity.

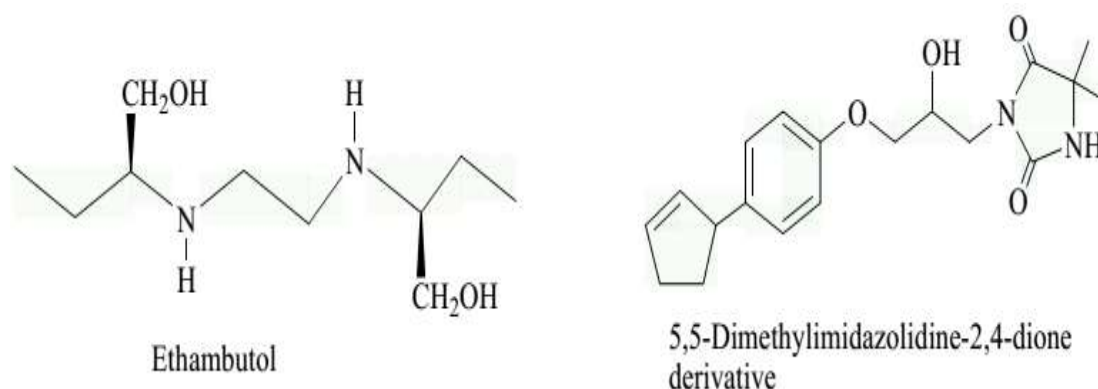


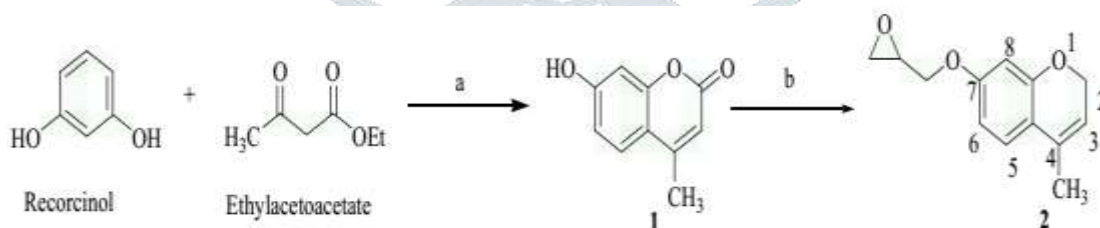
Figure – 2: Antibacterial agents

MATERIAL AND METHOD:–

A series of coumarinyl amino alcohols synthesized herein were prepared by the regio-selective opening of the oxirane ring in coumarinyl epoxide with different primary or secondary amines. All these compounds were evaluated for their antimicrobial activity against different Gram-positive and Gram-negative bacterial strains as well as fungi. The synthesized compounds are:

7-(3-cyclopropylamino-2-hydroxy-propoxy)-4-methyl-2H-1-benzopyran-2-one (3); 7-(3-cyclohexylamino-2-hydroxy-propoxy)-4-methyl-2H-1-benzopyran-2-one (4); 7-(2-hydroxy-3-piperidin-1-yl-propoxy)-4-methyl-benzopyran-2-one (5); 7-(2-hydroxy-3-morpholin-4-yl-propoxy)-4-methyl-2H-1-benzopyran-2-one (6); 7-(3-butylamino-2-hydroxy-propoxy)-4-methyl-2H-1-benzopyran-2-one (7); 7-(3-octylamino-2-hydroxy-propoxy)-4-methyl-2H-1-benzopyran-2-one (8); 7-(3-hexadecylamino-2-hydroxy-propoxy)-4-methyl-2H-1-benzopyran-2-one (10); 7-(2-hydroxy-3-oleylamino-propoxy)-4-methyl-2H-1-benzopyran-2-one (11); 7-(3-carboxypropylamino-2-hydroxy-propoxy)-4-methyl-2H-1-benzopyran-2-one (12).

The initial step of the synthesis of this class of molecules involved the classic Pechmann condensation of resorcinol with ethylacetoacetate to give 7-hydroxy-4-methylcoumarin 1 in good yield. Reaction of 1 with (R/S) epichlorohydrin in presence of K_2CO_3 under reflux condition provided oxiranyl coumarin 2. The completion of the reaction was monitored by TLC and during the reactions side products were also formed which could not be isolated in pure form. The synthesized compound was purified by column chromatography and was characterized using 1H NMR, ^{13}C NMR and mass spectroscopy.

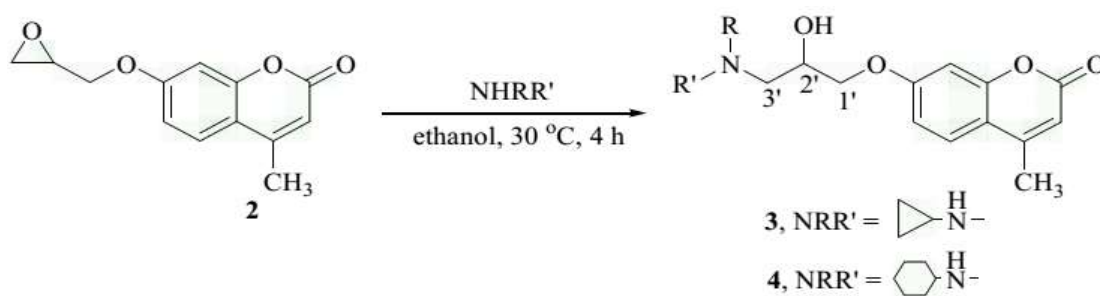


Scheme – 1: Regents and conditions: a. conc. H_2SO_4 , $0^\circ C$, 24^{th} ;

b. epichlorohydrin, K_2CO_3 , reflux

In the next sequence of reactions heterocyclic secondary amines were selected to synthesize 7-oxycoumarinyl aminoalcohol derivatives. Thus, oxiranyl coumarin (2) on reaction with piperidine and morpholine as above gave respective aminoalcohol derivatives 5 and 6 in good yields. The structures of these compounds were established on the basis of their spectral data and microanalysis. The ESI spectra of compounds 5 and 6 showed peaks at m/z 318 and 320

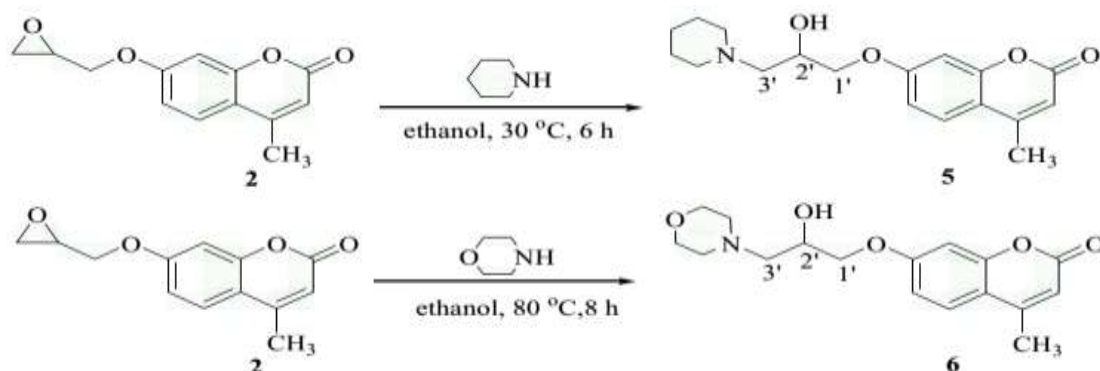
corresponding to their $[M+H]^+$, respectively. ^1H and ^{13}C NMR spectra of these compounds showed signal for benzopyrone ring protons and carbons at around their usual δ values as described earlier. However, in compound 5 the proton signals for the two methylenes of the piperidiny ring adjacent to $-\text{N}$ appeared as multiplet at δ 2.5 while other methylene protons were observed as m at δ 1.54 whereas in compound 6 methylene protons of the morpholine ring appeared as two multiplets each integrating for four protons at δ 4.06 and 2.67. In ^{13}C NMR spectrum the carbons of the piperidiny ring were observed at δ 54.6, 25.9 and 24.0. Similarly, carbon signals of the methylene carbons of the morpholine ring were observed at δ 66.8, 66.7, 53.7, 53.9 and 53.6, besides other carbon signals.



Scheme: 2

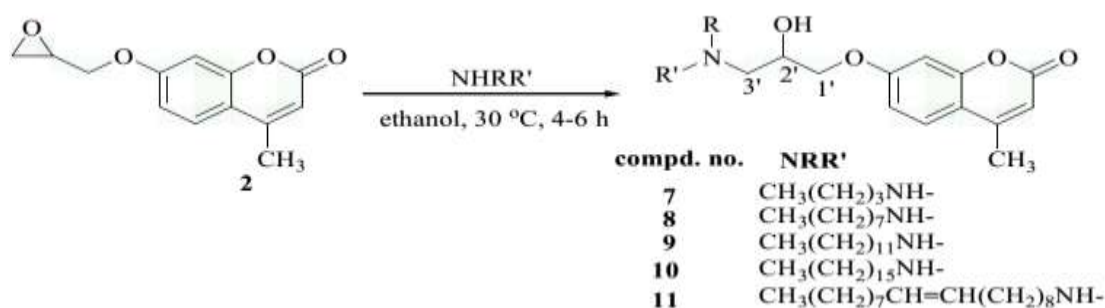
Similar reaction of the above coumarinyl epoxide separately as above with other acyclic primary amines cotylamine, dodecylamine, hexadecylamine and oleyl amines and isolation of the products by column chromatography using appropriate eluent resulted in respective coumarinyl amino alcohols 8, 9, 10 and 11 in good yields. Compounds 8, 9, 10 and 11 showed peaks at m/z 362, 418, 474 and 500 corresponding to their $[M+H]^+$ in their ESI spectra. In ^1H and ^{13}C NMR spectra of these compounds all the protons and carbons of the benzopyrone ring and propanol linker were observed at around their usual field strengths in accordance with the structures as explained above. The NCH_2 protons of the alkyl chain appeared as multiplet at around δ 3.7 in all these compounds along with $\text{H}-3'$ protons whereas NCH_2CH_2 protons were observed as multiplet at around δ 1.6. A triple at around δ 0.87 with J value of 6.6 Hz was attributed to the terminal methyl protons of octyl, dodecyl, hexadecyl and oleyl chains. However, in compound 11 the olefinic ($\text{CH}=\text{CH}$) protons of the oleyl chain appeared as two proton multiplet at δ 5.33 and the methylene protons at the carbon atom directly attached to the olefinic carbons ($\text{CH}_2\text{CH}=\text{CHCH}_2$) appeared as multiplet at δ 1.98 integrating for four protons. In ^{13}C NMR of the above compounds signal at around δ 40 was attributed to the methylene carbon of the alkyl chains attached to nitrogen; whereas terminal methyl carbon of the alkyl chains appeared at

around δ 14.0. Signals at δ 130.3 and 130.1 in compound 11 were accounted for olefinic carbons ($\text{CH}_2\text{CH}=\text{CHCH}_2$) of the oleyl chain.



Scheme: 3.

Finally the reaction of coumarinyl epoxide 2 with γ -amino butyric acid was undertaken in an order to see the effect of introduction of hydrophilic substituent on the bioprofile of the molecules. Thus reaction of 2 with γ -amino butyric acid at 80°C in ethanol led to the formation of compound 12 in moderate yield. ESI spectra of the above compound exhibited $(\text{M}+\text{H})^+$ peak at m/z 336. ^1H and ^{13}C NMR spectra of the compound exhibited proton and carbon signals corresponding to coumarin skeleton and propanol linker at around their usual δ values with the only difference of appearance of signals for protons and carbons corresponding to carboxypropyl substituent. Its ^1H NMR spectrum displayed signals for methylene protons ($-\text{CH}_2\text{CH}_2\text{COOH}$ and $-\text{CH}_2\text{COOH}$) of carboxypropyl substituent at δ 1.76 and 2.47 ($J = 7.1$ Hz), respectively, whereas methylene protons attached to nitrogen appeared as multiplet at δ 3.39 along with H-3' protons.



Scheme: 4.

RESULTS AND DISCUSSION:-

The synthesized coumarins 3–12 were screened for their in vitro antibacterial activities against *B. Subtilis* MTCC 441, lincimycin resistant *P. aeruginosa* MTCC 424, methicillin and ampicillin resistant *E. coli* MTCC 64 and *K. pneumoniae* MTCC 3884, ampicillin and vancomycin resistant *P. vulgaris* MTCC 426 and *S. aureus* MTCC 96. The synthesized compounds were also evaluated for antifungal efficacy against three fluconazole resistant strains *C. albicans* MTCC 1326, *C. terreus* MTCC 1716 and *S. cerevisiae* MTCC 307. The minimum

inhibitory concentration (MIC) of synthesized compound was determined by two-fold serial dilution method using clinically antimicrobial drugs erythromycin, novobiocin and amphotericin B as standards (NCCLS 2002). The MIC was defined as the lowest concentration of the tested compound at which no growth of the strain was observed in a period of time and under specified experimental conditions.

Table – 1: Antimicrobial activities of compound 3–12 expressed as MIC ($\mu\text{g/mL}$)

Compd	P. aeruginosa	E. coli	K. pneumoniae	P. vulgaris	S. aureus	B. subtilis	C. albicans	C. terreus	S. cerevisiae
3	>200	>200	75	>200	>200	>200	>200	>200	>200
4	>200	>200	50	>200	>200	>200	>200	>200	>200
5	>200	16	6.25	>200	>200	25	>200	>200	3.125
6	>200	100	>200	>200	>200	100	>200	>200	>200
7	>200	32	>200	>200	>200	>200	>200	>200	>200
8	50	>200	50	100	>200	>200	>200	>200	>200
9	1.09	25	6.25	6.25	>200	12.5	6.25	>200	6.25
10	1.95	>200	12.5	6.25	>200	>200	25	>200	12.5
11	50	>200	100	75	>200	>200	>200	>200	>200
12	>200	>200	75	>200	>200	>200	.25	>200	>200
ERY ^a	50	32	Resistant	75	0.195	3.125	—	—	—
NOV ^b	Resistant	30	25	Resistant	0.15	6.2	—	—	—
AMP ^c	—	—	—	—	—	—	12.5	6.25	12.5

^aReference drug : Erythromycin.

^bReference drug : Novobiocin.

^cReference drug : Amphotericin B.

From the above results it can be deduced that among all the synthesized coumarins, compounds 5, 9 and 10 provided good potency against the test bacterial and fungal strains. Out of the nine microbial strains examined in this study, 5 and 10 were active against five microbial strains while 9 exhibited activity against seven strains. Thus, compound 9 exhibiting uniformly good antimicrobial activity, with very low inhibitory concentration values against the Gram-negative bacteria *P. aeruginosa*, 1.09 $\mu\text{g/mL}$; *E. coli*, 25 $\mu\text{g/mL}$, *K. pneumoniae*, 6.25 $\mu\text{g/mL}$; *P. vulgaris*, 6.25 $\mu\text{g/mL}$ and Gram-positive bacterium *B. subtilis*, 12.5 $\mu\text{g/mL}$, was the most potent compound of the series. Compound 9 also showed considerable activity against fungal strains *C. albicans* and *S. cerevisiae* with MIC value of 6.25 $\mu\text{g/mL}$. Interestingly, compound 9 exhibited more potency against Gram-negative bacteria than the Gram-positive bacteria. It was 50- and 12-folds more active against *P. aeruginosa* and *P. vulgaris*, respectively, in comparison to erythromycin. Noticeably, both these bacterial strains were resistant to novobiocin. Moreover, compound 9 displayed better antifungal efficacy against *C. albicans* and *S. cerevisiae* than the standard drug amphotericin B. None of the compound of the series was active against fungal

strain *C. terreus*. However, substantial antifungal efficacy of coumarins 5, 9, 10 suggests that further optimization of these compounds may provide a new class of antifungal agents. From the bioactivity results, it was also established that long chain amines were more active than the short chain amines. However, the optimum length of alkyl chain with respect to antimicrobial activity was found to be twelve for this series, further increase (10 and 11) and decrease (7 and 8) in chain length from twelve carbon chain resulted in lowered activity. Introduction of cycloalkyl or some hydrophilic group like carboxypropyl as amino substituent also lowered the activity profile.

CONCLUSION:-

Present study concluded that a series of 7-oxycoumarinyl amino alcohols were designed and synthesized for the first time via an easy, convenient and efficient route. The antimicrobial results showed that some of the compounds showed moderate to excellent in vitro activity against different Gram –positive and Gram-negative bacterial strains and fungi with MIC values between 1.09 and 75 µg/mL. The preliminary SAR was discussed. Compounds 5, 9 and 10 exhibited better activity (MIC = 6.25-12.5 µg/mL) against Gram-negative bacterium *K. pneumoniae* than novobiocin and also elicited potent activity (MIC = 3.12-12.5 µg/mL) against fungal strain *S. cerevisiae*. Compounds 9 and 10 also demonstrated potent antibacterial activity against novobiocin resistant Gram-negative bacteria *P. aeruginosa* (MIC = 1.09 and 1.95 µg/mL, respectively) and *P. vulgaris* (MIC = 6.25 µg/mL). Compound 9 with twelve carbon chain length showed broad spectrum activity against five bacterial strains (MIC = 1.09-25 µg/mL) and two fungal strains (MIC = 6.25 µg/mL) and was proved to be the most active compound of the series. Thus compound 9 offers a fruitful scaffold for further investigations in finding leads with improved antimicrobial activity.

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