ANTIBACTERIAL ACTIVITY OF NEWLY SYNTHESISED PYRIDINYL COUMARIN DERIVATIVES

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Summary

Synthesis of Pyridinyl coumarins were achieved by treating 3- bromoacetyl coumarins with pyridine, esters of nicotinic and isonicotinic acids in anhydrous toluene as solvent system. Initially 15 derivatives of coumarinyl pyridinium bromides (**1.a – o**) were synthesised and purified in good yields. Three of the 3-coumarinoyl pyridinium bromide salts (**1.m – o**) reacted with different chalcones in glacial acetic acid to obtain 7 derivatives of pyridinyl coumarins (**2.a – g**). Further, 3-bromoacetyl coumarins treated with quinoline in dry toluene to obtain coumarinoyl quinolinium salts (**3.a – c**). Out of the 22 test compound synthesized, 8 test compounds exhibited minimum inhibitory concentration on par with the standard antibacterial such as Amoxicillin and Gentamycin. Test compounds showing promising antibacterial activity were showing Log P values well within the range to express the optimum biological activity.

Key words: Pyridinyl coumarin, 3-Bromoacetylcoumarin, Antibacterial

Introduction

Majority of the antimicrobial agents used in the treatment of infectious disease are antibiotics and chemotherapeutic agents. Rapid emergence of the multidrug resistance to antimicrobial agents in pathogenic bacteria has become a major threat in the treatment of infectious diseases [1]. The C_4-substituted aryloxymethyl, arylaminomethyl, and dichloroacetamido methyl coumarins, along with the corresponding 1-azacoumarins, have been demonstrated to be potential anti-microbial and anti-inflammatory agents [2-3].

Studies on pyridine-fused polycyclic coumarins have highlighted their potential as thymine dimer photosensitisers and the structurally related compounds of both coumarin and carbostyrils have also been found to act via the DNA gyrase pathway in their anti-bacterial activity [3]. Novobiocin possess coumarin nucleus, a known DNA gyrase inhibitor effective in breast cancer cells [4]. In the present work, we have synthesised newer pyridinyl coumarins and were screened for their antibacterial activity.

Materials and methods

The chemicals were of AR-grade and LR-grade obtained from Sigma-Aldrich, Sisco Research Laboratories, Qualigens, Rankem, S.D. Fine Chemicals, Hi-Media, Merck, Loba Chemicals and NICE. Melting point was determined using melting point apparatus (Shital Scientific industries) and were found uncorrected. The reactions were monitored by TLC and the R*f* values were determined using TLC plates with the solvent system; methanol: ethanol 6:1. λ_{max}, ϵ_{max} for the synthesized compounds were established by UV- visible spectrometer (Shimadzu UV-Vis Spectrophotometer UV-1650 PC) as explained in the literature [5]. The log P value was determined as it was explained in the literature [6-7]. The IR studies were done with FT-IR (Shimadzu FTIR 8310). NMR was carried out by the instrument NMR (AMX 400) and the mass spectral studies were done using Sciex 3000 LC-MS-MS (70 eV), Applied biosystems, Canada

Preparation of coumarinyl pyridinium bromide salts (1.a - o; scheme I)
A solution of 0.03 moles of 3-bromoacetylcoumarin derivatives in dry toluene was added to 0.0315 moles of pyridine / esters of nicotinic acid / isonicotinic acid and refluxed for 2 hr. Then set aside for 4 -5 hrs. The resultant salt was separated and washed with hot toluene. The crude product was recrystallized from ethanol: n-hexane and was dried.

Synthesis of pyridinyl coumarins (2.a - g; scheme I)
A solution of 0.03 moles of 3-coumarinoyl pyridinium bromide salts in glacial acetic acid, ammonium acetate were mixed to which a solution of 0.003 moles of chalcones in glacial acetic acid was added and the resulting mixture was stirred well at room temperature for 1 hr and then refluxed at 130°C for 6 hrs. Further, the reaction mixture was allowed to reach room temperature and was left overnight. It was then poured into ice-cold water and the product separated out was dried and was recrystallized from dimethylsulphoxide.

Synthesis of coumarinyl quinolinium bromide (3.a - c; scheme I)
A solution of 0.03 moles 3-bromoacetylcoumarin in dry toluene was added to 0.0315 moles of quinoline and refluxed for 2 hr. The solution was allowed to cool at room temperature for 4 -5 hr. The resultant salt was filtered, washed with hot toluene and recrystallized from ethanol.

Determination of qualitative antibacterial activity
All the twenty two synthesized test compounds were tested against four species of bacteria namely, *Bacillus subtilis* (gram positive) *Staphylococcus aureus* (gram positive) *Escherichia coli*, (gram negative) *Pseudomonas aeruginosa* (gram negative). Stock solutions of synthesized test compounds and standard drugs were prepared in dimethyl sulfoxide (DMSO). The test compounds were used at a concentration of 500µg/50 µl. Gentamycin and Amoxycillin were used as standards at a concentration of 10 µg /50 µl and at a concentration of 5µg/50 µl respectively. Mueller-Hinton agar medium was used for the agar diffusion

method (8-9). The inoculum was added to the medium and was poured into sterile petridishes for solidifying. Wells (bores) were made on the medium using sterile borer after solidification. 50 µl of the test and standard solutions were added to the respective bores. A control having only DMSO in the bore was maintained in each plate. The petridishes were kept at room temperature for 30 minutes for diffusion to take place and then was incubated at 37°C for 24 hr. The zone of inhibition was observed and measured using a scale. The test compounds exhibiting promising activity were then evaluated for their MIC using 96-well plate method.

Determination of minimum inhibitory concentration (MIC)

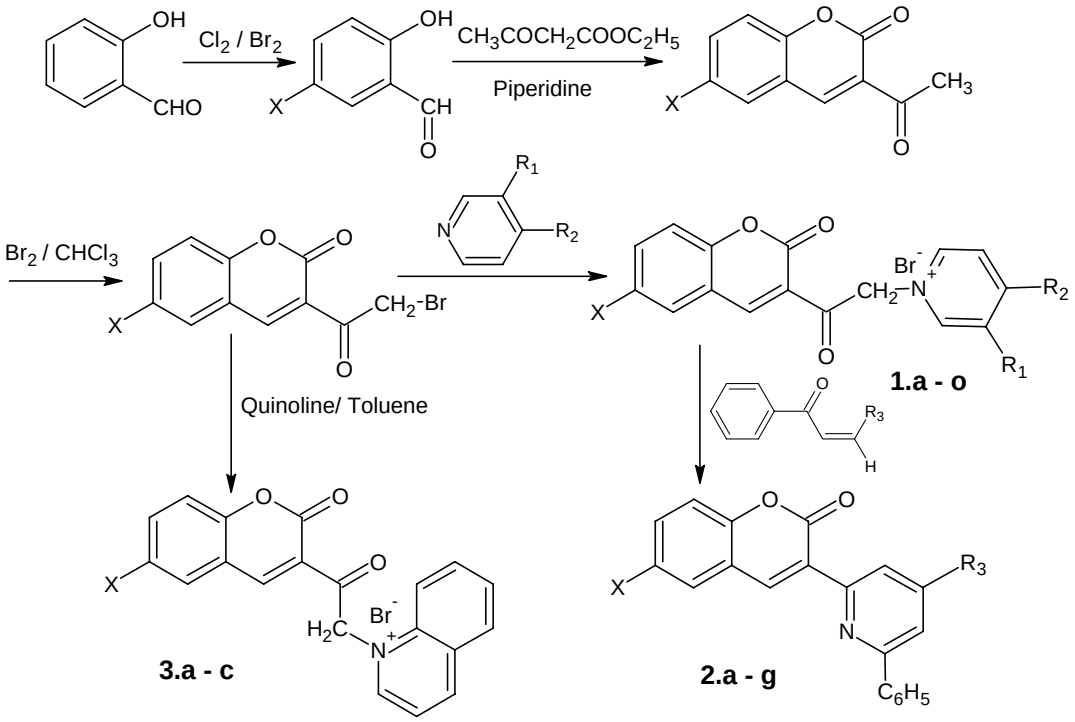
The cultures were then inoculated into Luria broth medium and incubated for 24 hr. The optical density (OD) of bacteria from mid log phase of growth was measured at 540 nm and diluted in fresh medium so as to get the OD of 0.004 (corresponding to $5x10^5$ CFU/ml). Graded concentrations (0.2-102.4 µg/50 µl) of the synthesized test compounds and the standards were exposed to 200µl of bacterial suspension for 24 and 48 hr [8-9]. The effect of drugs on growth of organism was monitored by measuring the optical density at 540 nm using ELISA microplate reader (Molecular Devises, USA). The MIC of the test compounds were determined by plotting the OD as a function of concentration. The above operation was carried out under aseptic condition in sterile area. Determinations of MIC were performed in triplicate.

Results and discussion

The starting material, 3-acetyl coumarin was synthesised from salicylaldehyde by treating with ethyl acetoacetate in the presence of piperidine as catalyst. This was purified by recrystallisation with glacial acetic acid. The yield of the final compound was 84%, with mp of 116°C, R*f* value at 0.70, the solvent used were benzene: chloroform 7:3. 3-acetyl coumarin on bromination in glacial acetic acid gave 3-bromoacetyl coumarin. It was further recrystallised from chloroform to give brownish needle shaped crystals. The purity of the compound was checked by melting point and TLC. Yield: 76%, mp: 165°C, R*f* value: 0.79 in the same solvent system. 3-bromoacetyl coumarin with pyridine in dry toluene gave 3-coumarinyl pyridinium bromide salt (**1.m**). Yield: 87%, R*f* value: 0.6 in the solvents system methanol: ethanol 6:1. The IR spectral data (Shimadzu FTIR 8700) of 3-coumarinyl pyridinium bromide (**1.m**) was 3435 (s, N-CH2str), 3087 (s, ArC-H str), 2923.9 (w, C-H str), 1713 (s, C=O str), 1598.9 (s, C=N str), 1550, 1492, 1473.5 (s, ArC=C str), 1245.9 (s, C-N str).

Presence of piperidine as catalyst gave 3-acetyl-6-bromocoumarin. It was recrystallised from the glacial acetic acid and then brominated in chloroform to give 3-bromoacetyl 6-bromocoumarin. Finally, the crude product was recrystallised from glacial acetic acid to give yellow needle shaped crystals. 3-bromoacetyl-6-bromocoumarin with pyridine in dry toluene gave 3-coumarinyl pyridinium bromide salt **(1.n).** Yield: 83%, R*f*: 0.61 with the solvents system methanol: ethanol 6:1. IR spectral data of 6-bromo-3-coumarinyl pyridinium bromide (**1.n**) was 3436.9 (s, N-CH_2 str), 3087.8 (s, ArC-H str), 2923.9, 2869.9 (w, C-H str), 1728.3 (s, C=O str), 1598.9 (s, C=N str), 1550.7, 1494.7, 1469.5 (s, ArC=C str), 1313.2 (s, C-N str), 680.8 (s, C-Br str).

Scheme I - Scheme synthesis of pyridinyl coumarin derivatives



Compounds	X	R_1	R_2	R_3
1.a	-H	-H	$-COOC_2H_5$	-
1.b	-Br	-H	$-COOC_2H_5$	-
1.c	-Cl	-H	$-COOC_2H_5$	-
1.d	-H	$-COOC_2H_5$	-H	-
1.e	-Br	$-COOC_2H_5$	-H	-
1.f	-Cl	$-COOC_2H_5$	-H	-
1.g	-H	-H	$-COOCH_3$	-
1.h	-Br	-H	$-COOCH_3$	-
1.i	-Cl	-H	$-COOCH_3$	-
1.j	-H	$-COOCH_3$	-H	-
1.k	-Br	$-COOCH_3$	-H	-
1.l	-Cl	$-COOCH_3$	-H	-
1.m	-H	-H	-H	-
1.n	-H	-H	-H	-
1.o	-H	-H	-H	-
2.a	-H	-	-	$-C_6H_5$
2.b	-Br	-	-	$-C_6H_5$
2.c	-Cl	-	-	$-C_6H_5$
2.d	-H	-	-	$-C_6H_4$-(2'-Cl)
2.e	-Br	-	-	$-C_6H_4$-(2'-Cl)
2.f	-Cl	-	-	$-C_6H_4$-(2'-Cl)
2.g	-H	-	-	- Furfuryl
3.a	-H	H	H	-
3.b	-Br	H	H	-
3.c	-Cl	H	H	-

Bromosalicyladehyde was prepared by bromination of salicyladehyde in glacial acetic acid and was recrystallised from aqueous ethanol. This on treatment with ethylacetoacete, in Salicyladehyde was chlorinated using potassium permanganate and concentrated HCl and the product was recrystallised from aqueous ethanol to give pure chlorosalicyladehyde. This on treatment with ethylacetoacetate in presence of piperidine as catalyst gave 3-acetyl 6-chlorocoumarin. R*f* value was at 0.66 in the solvent system benzene: chloroform 7:3, mp: 210°C. It was further recrystallised from glacial acetic acid and then brominated in alcohol free chloroform to 3-bromoacetyl-6-chlorocoumarin and recovered as greenish needle shaped crystals. Yield: 75%, R*f*: 0.79 (benzene: chloroform 7:3). 3-bromoacetyl-6-chlorocoumarin with pyridine in dry toluene gave 6-chloro-3-coumarinyl pyridinium bromide salt **(1.o).** IR data of 6-chloro-3-coumarinyl pyridinium bromide (**1.o**) was 3404 (s, N-CH_2 str), 3006 (s, ArC-H str), 2871 (w, C-H str), 1728 (s, C=O str), 1635 (s, C=N str), 1600, 1554, 1496 (s, ArC=C str), 1245 (s, C-N str), 761 (s, C-Cl str).

The purified parent compounds such as 3-bromoacetyl coumarin, 3-bromoacetyl 6-bromocoumarin and 3-bromoacetyl 6-chlorocoumarin were refluxed with ethyl and methyl isonicotinate and nicotinate in toluene to obtain twelve different pyridyl coumarin (scheme I, **1.a-l**). They were then recrystallised from ethanol: n-hexane. The physicochemical characteristics are given in the table 1. Their structures were established by IR spectral studies as shown in the table 2. Further, the structure of **1.a** and **1.c** was also supported by ^{1}H-NMR (300 MHz, AMX 400) and mass spectral study. The three parent 3-coumarinyl pyridinium bromide salts (**1.m-o**) were reacted with different chalcones in acetic acid medium to obtain seven different substituted pyridinyl coumarins (scheme I, **2.a-g**). The derivatives, **2.a-g** were further purified (yields 55 - 73%). The IR spectra of these compounds showed the absence of band at 3400 cm^{-1} for N-CH_2 stretch indicating for confirmation of final compound such as **2.a-g** as shown in table 2. Further, the structure of **2.a** was also supported by ^{1}H NMR and mass spectral studies. Similarly, the parent compounds 3-bromoacetyl coumarin, 3-bromoacetyl-6-bromo coumarin and 3-chloroacetyl-6-bromo coumarins were refluxed with quinoline in dry toluene to give corresponding coumarin quinolinium bromide salts (scheme I, **3.a-c**) at yield 75 – 80%. Structures of these compounds were confirmed by IR spectral data as shown in table 2.

Out of 22 test compounds synthesized and purified, the halogen substituted coumarins possessed greater influence on partition coefficient than the unsubstituted compounds. Electronegativity of bromide atom resulted in higher Log P value than that of chloro substituted compounds and unsubstituted compounds. Test compounds **3.a-c** was found to have higher values compared to that of corresponding **1.a-l**. The pKa of the synthesized compounds **1.a-l**, **2.a-g** and **3.a-c** were in the range of 6 to 6.5, is necessary for the ionization at gastric pH and unionization at intestinal pH. λ_{max} and ϵ_{max} were found in the range of 205-226 and 1025 – 1297 respectively as shown in table 1. λ_{max}, ϵ_{max} and pKa were established by UV- visible spectrometer.

Table 1: Physicochemical properties of the synthesised compounds

Compound	Molecular formula	Mol Wt	Yield (%)	mp (°C)	R*f* value*	Log P	λ_{max}	ϵ_{max}	pKa
1.a	$C_{19}H_{16}NO_5Br$	418	85	210	0.80	0.54	210.5	1088	6.25
1.b	$C_{19}H_{15}NO_5Br_2$	497	75	228	0.81	0.83	226.0	1025	6.30
1.c	$C_{19}H_{15}NO_5ClBr$	452.5	78	220	0.79	0.96	221.0	1032	6.32
1.d	$C_{19}H_{16}NO_5Br$	418	75	224	0.72	1.32	214.5	1050	6.38
1.e	$C_{19}H_{15}NO_5Br_2$	497	79	225	0.72	1.60	227.0	1124	6.30
1.f	$C_{19}H_{15}NO_5ClBr$	452.5	81	226	0.81	1.40	219.0	1275	6.18
1.g	$C_{18}H_{14}NO_5Br$	404	83	200	0.74	1.20	213.0	1287	6.29
1.h	$C_{18}H_{13}NO_5Br_2$	483	78.5	208	0.72	1.23	215.0	1123	6.16
1.i	$C_{18}H_{13}NO_5BrCl$	438.5	82	218	0.80	1.21	214.0	1125	6.20
1.j	$C_{18}H_{14}NO_5Br$	404	79	218	0.68	1.09	219.0	1212	6.22
1.k	$C_{18}H_{13}NO_5Br_2$	483	83	221	0.70	1.08	210.0	1213	6.29
1.l	$C_{18}H_{13}NO_5BrCl$	438.5	76	227	0.76	1.01	206.0	1162	6.30
2.a	$C_{26}H_{17}NO_2$	375	62	188	0.79	1.04	205.0	1397	6.31
2.b	$C_{26}H_{16}NO_2Br$	454	62.5	190	0.82	1.18	206.0	1389	6.22
2.c	$C_{26}H_{16}NO_2Cl$	409	72.3	191	0.80	1.20	205.0	1132	6.25
2.d	$C_{26}H_{16}NO_2Cl$	409	65	191	0.81	1.31	205.0	1112	6.26
2.e	$C_{26}H_{15}NO_2ClBr$	488.5	55	191	0.82	1.41	204.0	1123	6.28
2.f	$C_{26}H_{15}NO_2Cl_2$	444	73	200	0.78	1.30	206.5	1102	6.30
2.g	$C_{26}H_{15}NO_3$	365	56.5	189	0.76	1.20	213.0	1287	6.29
3.a	$C_{20}H_{14}NO_3Br$	396	79.8	230	0.50	0.95	240.0	1065	6.31
3.b	$C_{20}H_{13}NO_3Br_2$	475	76.5	219	0.74	1.20	250.0	1000	6.18
3.c	$C_{20}H_{13}NO_3BrCl$	430	75	220	0.76	1.20	246.0	1007	6.09

Recrystallised from ethanol: n-hexane; * methanol: ethanol 6:1

^{1}HNMR and Mass spectral data of some selected compounds

3-coumarinoyl (4'-ethoxy carbonyl) pyridinium bromide (1.a): ^{1}H NMR 300 MHz, $CDCl_3$: δ 1.5 (2H, t, H-α-CH_2CH_3), 4.53 (3H, q, β-CH_2CH_3), 7.13 (2H, d, CH_2), 7.28 (1H, s, H-5), 7.62 (1H, t, H-7), 7.71 (2H, d, H-3'/5'), 8.50 (2H, m, H-6/8), 8.77 (1H, s, H-4), 9.91 (2H, d, H-2'/6'); Mass: m/e 418; MF: $C_{19}H_{16}NO_5Br$; mw: 418

6-chloro-3-coumarinoyl (4'-ethoxy carbonyl) pyridinium bromide (1.c): ^{1}H NMR 300 MHz, $CDCl_3$: δ 1.47 (2H, t, H-α-CH_2CH_3), 4.55 (3H, q, β-CH_2CH_3), 6.79 (2H, s, CH_2), 7.42 (1H, d, H-8), 7.70 (1H, m, H-7), 7.89 (1H, s, H-5), 8.55 (2H, d, H-3'/5'), 8.62 (1H, s, H-4), 9.59 (2H, d, H-2'/6'); Mass: m/e 451.9; MF: $C_{19}H_{15}NO_5ClBr$; mw:452.5

3-[4'-(2-chlorophenyl)-6'-phenyl pyridine-2'-yl] coumarin (2.d): ^{1}H NMR 300 MHz, $CDCl_3$: δ 7.35 (2H, s, Ar-H), 7.45 (4H, m, Ar-H), 7.55 (1H, m, Ar-H), 7.6 (5H, m, Ar-H), 7.73 (1H, d, Ar-H), 7.88 (1H, s, Ar-H), 8.15 (1H, d, Ar-H), 9.4 (1H, s, Ar-H); Mass: m/e 409; MF: $C_{26}H_{16}NO_2Cl$; mw: 409

Table 2: IR Spectral data

Comp	IR stretching							
	N-CH_2	ArC-H	C-H	C=O	C=N	C=C	C-N	Halogen
1.a	3402	3022	2958, 2927	1730, 1687	1610	1552, 1487, 1448	1247	-
1.b	3425	3001	2933, 2856	1722, 1641	1602	1550, 1487, 1498	1251	675 (C-Br)
1.c	3407	3006	2906	1735, 1703	1612	1560, 1463, 1417	1292	752 (C-Cl)
1.d	3408	3008	2933	1730, 1658	1600	1558, 1496	1229	-
1.e	3410	3001	2933	1733, 1652	1604	1552, 1498, 1471	1251	675 (C-Br)
1.f	3400	3014	2929	1730, 1693	1604	1552, 1498, 1471	1251	675 (C-Cl)
1.g	3396	3051	2925	1728	1604	1556, 1452	1292	-
1.h	3410	3014	2926	1733, 1695	1604	1552, 1452	1242	675 (C-Br)
1.i	3415	3006	2950	1737	1608	1556, 1461	1296	752 (C-Cl)
1.j	3433	3000	2923	1728, 1687	1602	1558, 1498, 1448	1305	-
1.k	3435	3003	2922	1732, 1681	1600	1548, 1471	1300	661 (C-Br)
1.l	3431	3089	2983, 2923	1726, 1689	1643	1602, 1557, 1446	1255	742 (C-Cl)
2.a	-	3043	2928	1687	1589	1542, 1496, 1452	1250	-
2.b	-	3058	2826	1687	1587	1544, 1494, 1474	1263	663 (C-Br)
2.c	-	3035	2923	1695	1590	1542, 1477, 1473	1265	758 (C-Cl)
2.d	-	3035	2935	1660	1590	1544, 1477, 1450	1265	758 (C-Cl)
2.e	-	3043	2922	1675	1606	1548, 1477	1280	746 (C-Cl)
2.f	-	3035	2983	1697	1596	1542, 1477	1250	752 (C-Cl)
2.g	-	3062	2950	1683	1602	1542, 1452	1280	-
3.a	3405	3000	2825	1695	1590	1554, 1446	1247	-
3.b	3373	2979	2881	1699	1596	1550, 1473	1247	655 (C-Br)
3.c	3408	2977	2881	1693	1600	1552, 1527, 1473	1249	746 (C-Cl)

Antibacterial activity: Qualitative antibacterial activity was determined by agar diffusion method using 100µg/ml Amoxycillin and Gentamycin as standard. Stock solutions of the test compounds were prepared in DMSO at a concentration of 100µg/ml, at which DMSO by itself did not exhibit any antibacterial activity. The zone of inhibition obtained was compared with that of standard. Compounds **1.a, 1.c, 1.d** and **1.i**, showed activity against gram positive and gram negative bacteria. Their zone of inhibition was 28, 24, 28 and 24mm respectively against *Bacillus subtilis*; at 30, 22, 26 and 32mm respectively against *Staphylococcus aureus*; at 14, 30, 26 and 24mm respectively against *Escherichia coli*; at 26, 22, 28 and 22 mm respectively against *Pseudomonas aeruginosa* as shown in the table 3. Compounds **1.j** and **1.k** showed activity against gram positive bacteria *Staphylococcus aureus*. They showed the zone of inhibition 26 mm each. Compounds from **2** series, such as **2.b, 2.d, 2.f** and **2.g** showed activity against both gram positive and gram negative bacteria. The zone of inhibition was at 36, 22, 26 and 30mm respectively against *Bacillus subtilis*; at 32, 24, 24 and 24mm respectively against *Escherichia coli*; at 36, 36, 30 and 22 mm respectively for *Staphylococcus aureus*; at 28, 24, 22 and 22 mm respectively against *Pseudomonas aeruginosa*. Compounds from the series **3**, such as **3.b** and **3.c** showed activity against gram positive bacteria. The zone of inhibition was at 20 mm each for *Bacillus subtilis*; 29 and 24 mm against *Staphylococcus aureus.*

Table 3: Antibacterial Activity: Zone of inhibition

Compound code	Zone of Inhibition (mm)			
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
1.a	28	14	26	30
1.b	-	-	-	-
1.c	24	30	22	22
1.d	28	26	28	26
1.e	-	-	-	-
1.f	-	-	-	-
1.g	-	22	-	-
1.h	-	-	-	26
1.i	24	24	22	32
1.j	26	-	-	22
1.k	26	-	-	22
1.l	-	26	28	24
2.a	-	-	-	-
2.b	36	32	28	36
2.c	30	-	-	30
2.d	22	24	24	36
2.e	18	-	-	26
2.f	26	24	22	30
2.g	30	24	22	22
3.a	-	-	-	-
3.b	20	-	-	29
3.c	20	-	-	24
Amoxicillin	34	33	34	36
Gentamycin	36	34	37	38

Out of 22 test compound screened for the qualitative antibacterial activity, eight compounds showed promising activity and were selected for quantitative antibacterial activity. The minimum inhibitory concentrations (MIC) of test compounds against the microbial organism were determined by 96 well plates by two fold dilution technique using ELISA reader. Amoxycillin and Gentamycin at a concentration ranging from 0.2-102.4µg/50µl were used as standard. The test compounds such as **1.a**, **1.c, 1.d** and **1.i** showed the MIC at 6.4, 6.4, 3.2 and 6.4 µg/50 µl respectively against *Bacillus subtilis*. The test compounds **2.b, 2.d**, **2.f** and **2.g** showed the MIC at 3.2, 6.4, 3.2 and 3.2 µg/50 µl respectively against *Bacillus subtilis*. The test compounds such as **1.a, 1.c, 1.d** and **1.i** showed MIC at 25.6, 12.8, 12.8 and 12.8 µg/50 µl respectively against *Eschirichia coli*. The test compounds such as **2.b, 2.d**, **2.f** and **2.g** showed the MIC at 25.6, 6.4, 6.4 and 6.4 µg/50 µl respectively for *Escherichia coli*. The test compounds **1.a, 1c, 1.d** and **1.c** showed the MIC at 3.2, 12.8, 6.4 and 3.2 µg/50 µl respectively against *Staphylococcus aureus*. The test compounds such as **2.b, 2.d, 2.f** and **2.g** showed the MIC at 3.2, 3.2, 3.2 and 12.8 µg/50 µl respectively against *Staphylococcus aureus*. All MIC data are represented in table 4.

Table 4: MIC of the few synthesised compounds

Compound	MIC (µg/50µl)	% inhibition compared to Gentamycin	% inhibition compared to Amoxicillin
Against *Bacillus subtilis*			
1.a	6.4	50	25
1.c	6.4	50	25
1.d	3.2	100	50
1.i	6.4	50	25
2.b	3.2	100	50
2.d	6.4	50	25
2.f	3.2	100	50
2.g	3.2	100	50
Amoxicillin	1.6		
Gentamycin	3.2		
Against *Escherichia coli*			
1.a	25.6	12.5	100
1.c	12.8	25	200
1.d	12.8	25	200
1.i	12.8	6.25	200
2.b	25.6	12.5	100
2.d	6.4	50	400
2.f	6.4	50	400
2.g	6.4	50	400
Amoxicillin	25.6		
Gentamycin	3.2		
Against *Staphylococcus aureus*			
1.a	3.2	25	50
1.c	12.8	6.25	12.5
1.d	6.4	12.5	25
1.i	3.2	25	50
2.b	3.2	25	50
2.d	3.2	25	50
2.f	3.2	25	50
2.g	12.8	6.25	12.5
Amoxicillin	1.6		
Gentamycin	0.8		

Conclusions

Out of the 22 compound synthesized, test compounds such as **1.a, 1.c, 1.d, 1.i, 2.b, 2.d, 2.f** and **2.g** exhibited antibacterial activity on par with that of the standard. Their Log P values were well within the range to express the optimum biological activity. An attempt could be made to understand the molecular mechanism of their antibacterial action. Coumarinyl test compounds showing antibacterial activity having quinoline, nicotinyl and isonicotinyl group could also be tested for their antimalarial, antiamoebic and antitubercular activity respectively.

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