

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF ACTIVITY OF NOVEL N- SUBSTITUTED 4 METHYL 5,7 DI HYDROXYL COUMARIN AND ITS ESTER DERIVATIVES

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ABSTRACT

Some novel N- Substituted 4 methyl 5,7 di hydroxyl coumarins were synthesized efficiently in two methods general synthesis and microwave method in 4 steps. Both the methods 12 compounds were synthesized. The synthesized compounds were identified by IR, NMR spectroscopic techniques. The present investigation deals with the synthesized compounds possessing good antibacterial activity. The antibacterial activity of the test compounds were determined by Kirby Bauer disc diffusion susceptibility test. In *Staphylococcus aureus* Gram (+) ve bacteria the antibacterial activity of the test compounds showed comparable activity as that of the standard. Among the tested compound V, VI, VII showed maximum activity while compounds VIII showed better activity and compound I, II, III showed less activity. In *Pseudomonas* Gm (-) ve bacteria the antibacterial activity of the test compounds showed comparable activity as that of the standard. Among the tested compound V and VIII showed maximum activity while compounds I, IV and VI showed better activity and compound II, III, VII showed minimum activity.

Keywords: Antibacterial activity, Novel N-Substituted 4 methyl 5,7 dihydroxyl coumarin derivatives.

INTRODUCTION

Coumarins and its derivatives have been proved as useful precursors for the synthesis of variety of medicinal agents¹. The heterocycles derived from these have also been tested for their anti-HIV, anti-inflammatory, anti-convulsant, antioxidant, antibacterial, antifungal, anti-carcinogenic and anti-histaminic activities^{2,3}. On the basis of our observation the present research work was carried out to synthesis some coumarin derivatives and further evaluate the antibacterial activity. In vitro antimicrobial activity of all synthesized compounds and standard drugs against 4 strains of bacterial culture which include 2 Gram + bacterial culture and 2 Gram- bacterial culture. The compounds show net enhancement activity

on coordination of metals with ligand but moderate activity as compared to standard drugs⁴.

Scheme^{5,6,7,8}

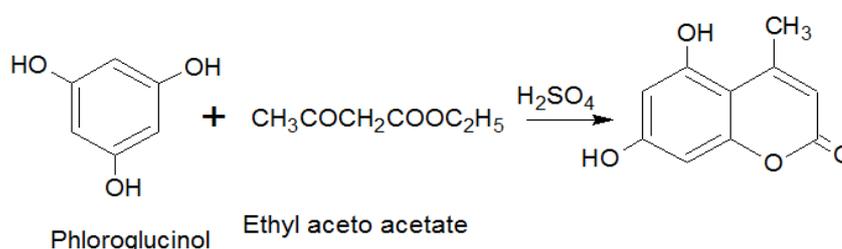
GENERAL SYNTHESIS

STEP 1: SYNTHESIS OF 4-METHYL 5, 7 DIHYDROXY COUMARIN

Placed 100 ml of concentrated sulphuric acid in 500 ml 3 necked flask fitted with thermometer and dropping funnel. Immersed the flask in an ice bath, and the temperature was maintained below 10°C. 0.1 mole of the Phloroglucinol in 0.103 mole of Ethyl acetoacetate was added drop wise with stirring. The temperature was maintained below 10°C by means of ice salt bath during the addition (1-2 hours). The reaction mixture

was kept at room temperature for 18 hours and then passed it with vigorous stirring into a mixture of 500 gm of crushed ice and 1 liter of water. The precipitate was collected by suction filtration and washed it three times with cold water. Dissolved the crude product of coumarin

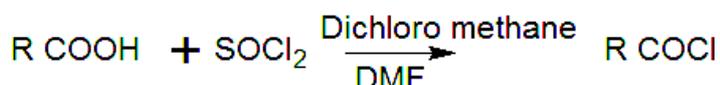
obtained in 5% of sodium hydroxide and checked the P.H. 10% HCl was added to this solution for the maximum precipitation to occur. Filtered and collected the pure recrystallised coumarin.



STEP 2: SYNTHESIS OF ACID CHLORIDE

In a 250 ml distilling flask fitted with a separating funnel was placed 0.1 mole of the corresponding acid dissolved in 10-20 ml of dichloromethane. The side arm of this flask was attached to a long downward condenser, which in turn was connected by means of an adapter to a 500 ml filter flask. A calcium chloride drying tube was joined by short piece rubber tubing to the tubulature of the filter flask. While the flask containing the acid was heated on a water bath which was kept at 60°C-65°C, a 20 ml portion of freshly distilled

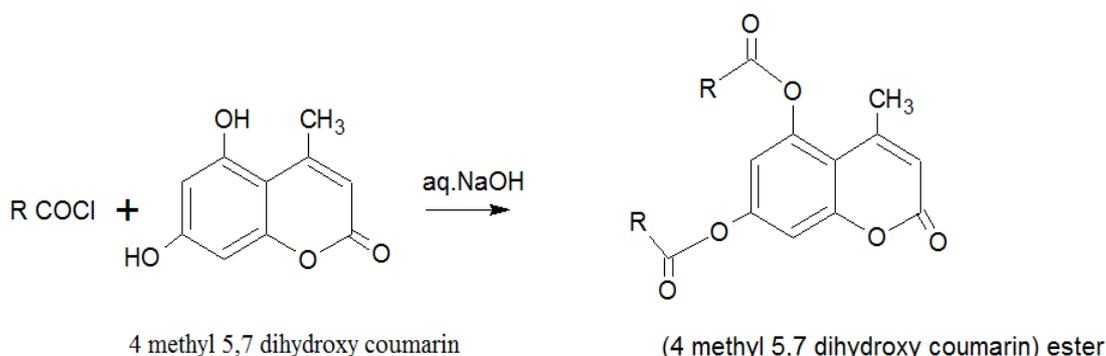
thionyl chloride was gradually added from the separating funnel. Two to three drops of dimethyl formamide was added. The temperature of the water bath was maintained at 60°C-65°C for 2-3 hours until the vigorous evolution of gas had nearly ceased. As soon as the reaction had been completed the separatory funnel was replaced by a thermometer and the excess thionyl chloride was carefully recovered by distillation. The acid chloride was next distilled over at 208-209°C and was collected rejecting first few drops.



STEP 3: SYNTHESIS OF COUMARIN BASED ESTERS

Dissolved about 1.5 gm of crude coumarin obtained in step: 1 in 10-15 ml of 10% aqueous sodium hydroxide in a round bottom flask. To this solution, 2.4 equivalent of acid

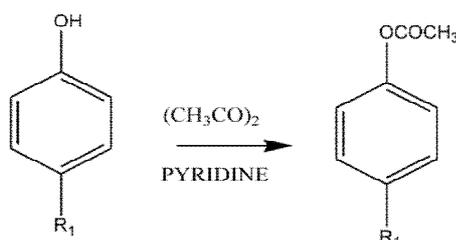
chloride was added at reduced temperature using an ice bath. The flask was shaken for half an hour and the content was filtered. The coumarin based ester prepared was recrystallised from ethanol.



MICROWAVE SYNTHESIS**STEP 1: PROCEDURE FOR ACETYLATION**

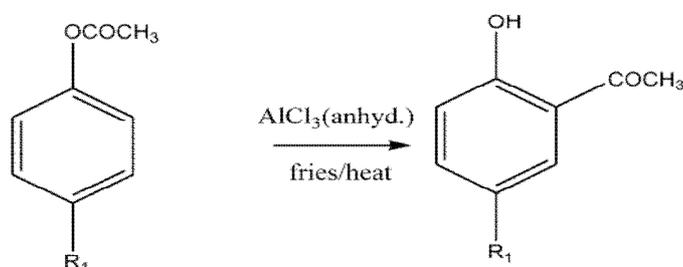
A completely dried two necked round bottom flask was taken. Added substituted phenol (0.1 equivalents), acetic anhydride (0.4 equivalents) and pyridine (10 ml). The lid was closed and the flask was placed in an unmodified catalyst microwave oven at the power setting (Level 7, 80% power, 455 watts output) for 6 minutes. The TLC was checked after 6 minutes to ensure the completion of the reaction. After the completion of the reaction

the reaction mixture was allowed to cool for some time. The cooled mixture was extracted with water: ethyl acetate mixture thrice. The organic layer was removed and some quantity of sodium sulphate was added to remove any traces of water and the solution was filtered in a dry round bottom flask. This organic layer was concentrated by distillation. Since the intermediate obtained was semisolid, it was not possible to find out the practical yield at this step.

**STEP 2: PROCEDURE FOR THE SYNTHESIS OF 2-SUBSTITUTE HYDROXY PHENONES**

Removed a little quantity of the intermediate from step: 1 in a small vial and added small amount of ethyl acetate for TLC purpose. 0.1 equivalents of Aluminium chloride was taken into a beaker along with 15 ml CCl_4 . The intermediate compound obtained after step: 1 was taken along with a little quantity of CCl_4 and this intermediate was added into a beaker containing Aluminium chloride drop wise with continuous stirring. The flask was kept in ice cold condition. After addition the reaction mixture was exposed to the microwave oven at the power settings (Level 3, 245 output

watt) under mild intensity. The intensity was increased very slowly for power level 3 to 9 each for 4 minutes. The TLC was checked to ensure the completion of the reaction. After the completion of the reaction, the entire mixture was poured into a round bottom flask and the CCl_4 was distilled and cooled. The mixture of water: 6N HCL (50:50) was prepared and the mixture was added very slowly into the cooled reaction mixture. The reaction mixture was extracted with Ethyl acetate thrice. The organic layer was removed in a dried beaker. Sodium sulphate was added into it and was filtered. The solvent was concentrated by distillation. The crude mixture of the product was obtained.

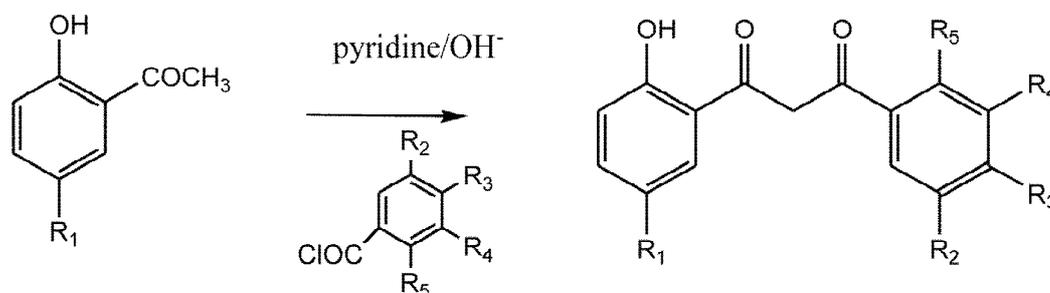
**STEP 3: INCORPORATION OF THE ACID CHLORIDE**

The substituted/unsubstituted acid chloride (0.2 equivalents) was taken in 10 ml of pyridine into a round bottomed flask and

added substituted or unsubstituted 2-hydroxyacetophenones obtained at the end of step: 2 into it. Powdered Sodium hydroxide (0.2 equivalents) was taken and added it to the above flask. Refluxed gently the reaction

mixture in the microwave oven in the power setting (Level 4, 40% power, 280 watts) for 5 minutes. The completion of the reaction was checked by TLC. The reaction mixture was cooled and poured into excess of 6N HCL.

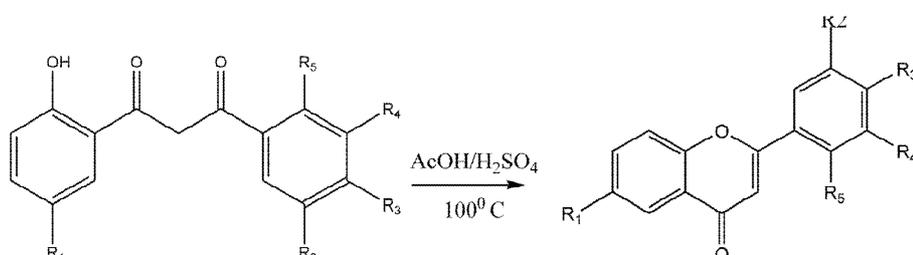
Yellow precipitate was obtained. The precipitate was filtered, washed with water and dried. Recrystallized the crude material with ethanol.



STEP 4: CYCLIZATION REACTION

The intermediate obtained at the end of step: 3 was taken along with acetic acid (10 ml) and concentrated Sulphuric acid in a beaker. Refluxed the reaction mixture in the microwave oven at the power settings (Level 8, 70% power, 490 watts) for 5 minutes. The

completion of the reaction was ensured using TLC. The reaction mixture was cooled and poured into crushed ice. Precipitate was obtained. Filtered off the resulting precipitate, washed it with water and dried. Recrystallized with ethanol: water. The product was dried, weighed and the practical yield was noted.



Synthesised Compounds

The synthesised compounds are in general synthesis in Scheme 1 derivative is 4-methyl 5, 7 dihydrocoumarin. In Scheme 2 derivative Toluene 4-sulphonyl chloride, 3, 4 dimethoxy benzoyl chloride, 4-chloro benzoyl chloride, Toluyl chloride, Benzoyl chloride, Acetyl chloride, 1-naphthyl acetyl chloride, P-methoxy benzoyl chloride. In Scheme 3 derivative 4-methyl 2-oxo 2H chromene 5, 7 diylbis toluene 4- sulphonate compound I, 4-methyl 2-oxo 2H chromene 5, 7 diylbis (dimethoxy benzoate) compound II, 4-methyl 2-oxo 2H chromene 5, 7 diylbis (chloro

benzoate) compound III, 4-methyl 2-oxo 2H chromene 5, 7 diyl ditoluate compound IV, 4-methyl 2-oxo 2H chromene 5, 7 diyl dibenzoate compound V, 4-methyl 2-oxo 2H chromene 5, 7 diyl diacetate compound VI, 4-methyl 2-oxo 2H chromene 5, 7 diyl bis (methoxybenzoate) compound VII, 4-methyl 2-oxo 2H chromene 5, 7 diyl bis (naphthyl acetate) compound VIII. In microwave synthesis derivatives of acid chlorides synthesized were Acetyl chloride, Benzoyl chloride, Salicylyl chloride. In derivatives of Coumarin synthesized were 1-benzo 2-phenyl pyran-3-one compound IX, 1-benzo 2-salicylyl pran-3-one compound X, 1-

nitrobenzo-2-phenyl pyran-3-one compound XI, 1-nitrobenzo-2-phenyl Salicylyl pyran-3-one compound XII.

Experimental^{9,10,11,12,13}

COMPOUND-I

4-Methyl 2-oxo 2-H chromene 5,7diylbis (toluene sulphonate).

IR Spectra

1077.87 (C-O-C stretching), 3153.40 (C=O stretching), 1621.68 (C=C stretching), 2958.04 (C-H stretching), 1112.60 (S=O stretching), 914.76 (C-O stretching).

NMR Spectra

2.291 (3H, Aliphatic protons), 7.136-7.116 (8H, Aryl protons), 7.491-7.471(3H,3,6,8 Aryl protons),2.531-2.485(6H, Aliphatic protons).

COMPOUND-II

4-Methyl 2-oxo 2-H chromene 5,7diylbis (dimethoxy benzoate).

IR Spectra

1137.94 (C-O-C stretching), 3422.59 (C=O stretching),1678.02 (C=C stretching),1466.55(CH bending), 1024.95 (C-O stretching),1599.84 (carboxylate), 2851.76 (OCH₃,CH stretching).

NMR Spectra

2.513-2.505(Aliphatic protons), 7.057-7.036(3H,2,5,6 Aryl protons), 3.479 (Aliphatic protons),7.434-7.430(3H,3,6,8 Aryl protons),7.578-7.553(Aryl protons).

COMPOUND-III

4-Methyl 2-oxo 2-H chromene 5, 7 diylbis (chloro benzoate).

IR Spectra

1141.23 (C-O-C stretching), 1602.87 (C=O stretching), 2927.07 (C-H stretching), 1254.61 (carboxylate stretching), 846.79 (C-Cl stretching), 1088.81(C-O stretching).

NMR Spectra

7.583-7.441(4H,2,3,5,6 Aryl protons), 2.513-2.505 (3H,Aliphatic protons),7.698-7.731(4H,Aryl protons),7.931-7.953(3H,3,6,8 Aryl protons).

COMPOUND-IV

4-Methyl 2-oxo 2H chromene 5, 7 diylditoluate.

IR Spectra

1128.28 (C-O-C Stretching), 1675.70 (C=O Stretching), 2962.10 (C-H Stretching), 1675.70 (C=C Stretching), 1112.28 (C-O Stretching).

NMR Spectra

2.371 (3H,Aliphatic protons), 7.312-7.293 (4H,3,4,5,6 Aryl protons), 2.505 (3H,Aliphatic protons), 7.845-7.824 (3H, 3,6,8 Aryl protons).

COMPOUND-V

4-Methyl 2-oxo 2H chromene 5, 7 diyldibenzoate.

IR Spectra

1138.58 (C-O-C Stretching), 1607.86 (C=O Stretching), 1440.60 (C-H bending), 1640.60 (C=C Stretching),935.02 (C-O Stretching).

NMR Spectra

7.793-7.454 (5H, 2,3,4,5,6 Aryl protons), 2.443 (3H, Aliphatic protons), 7.957-7.936 (5H, Aryl protons), 8.148-8.236(3H, 3,6,8 Aryl protons).

COMPOUND-VI

4-Methyl 2-oxo 2H chromene 5, 7 diyldiacetate.

IR Spectra

1600.38 (C=O Stretching), 1161.05 (C-O-C Stretching), 1679.58 (C=C Stretching), 2872.33 (C-H Stretching), 836.58 (C-O Stretching).

NMR Spectra

2.504-2.500 (3 H, Aliphatic protons), 6.686 (3H,3,6,8,Aromatic protons), 2.670-2.650 (3H, Aliphatic protons), 2.587 (3H, Aliphatic protons).

COMPOUND-VII

4-Methyl 2-oxo 2H chromene 5,7diylbis (naphthyl acetate).

IR Spectra

3424.21 (C=O Stretching), 2962.89 (C-H Stretching), 1290.95 (Carboxylate), 1611.65 (C=C Stretching), 841.94 (C-O Stretching), 1136.00 (C-O-C Stretching).

NMR Spectra

2.624-2.383 (3H, Aliphatic protons), 7.603-7.406 (7H, 2,3,4,5,6,7,8 Aryl protons), 7.622 (3H, 3,6,8 Aryl protons),8.171 (7H, Aryl protons).

COMPOUND-VIII

4-Methyl 2-oxo 2H chromene 5, 7 diylbis (methoxy benzoate).

IR Spectra

1687.06(C=C Stretching), 2959.13 (C-H Stretching), 927.07 (C-O Stretching), 1604.99 (C=O Stretching), 1166.94 (C-O-C Stretching), 2815.23 (C-H, -OCH₃Stretching), 1427.53 (Carboxylate).

NMR Spectra

2.502 (3H, Aliphatic protons), 7.029-7.007 (4 H, 2,3,5,6 Aryl protons), 7.159-7.137 (4H, Aryl protons), 7.902-7.880 (3H, Aryl protons), 3.823 (3H, Aliphatic protons), 3.887 (3H, Aliphatic protons).

COMPOUND-IX

1-benzo 2-phenyl pyran 3-one.

IR Spectra

2850.68 (C-H Stretching), 1623.19 (C=C Stretching), 1634.77 (C=O Stretching), 1030.03 (C-O-C Stretching), 857.17 (C-C Stretching).

COMPOUND-X

1-benzo 2-salicylyl pyran 3-one.

IR Spectra

3228.03 (OH Stretching), 2842.48 (C-H Stretching), 1625.67 (C=C Stretching), 853.03 (C-C Stretching), 1684.30 (C=O Stretching), 1092.73 (C-O-C Stretching).

COMPOUND-XI

1-nitrobenzene 2-phenyl pyran 3-one.

IR Spectra

1676.31 (C=C Stretching), 1420.40 (C-H bending), 1499.22 (Nitro stretching), 1070.69 (C-O-C Stretching), 881.84 (C-C Stretching), 1600.42 (C=O Stretching).

COMPOUND-XII

1-nitrobenzene 2-salicylylpyran 3-one.

IR Spectra

1320.13 (C-H bending), 1497.72 (Nitro stretching), 1609.74 (C=O Stretching), 1152.40 (C-O-C Stretching), 879.42 (C-C Stretching), 3448.15 (OH Stretching).

MATERIALS AND METHODS

Melting points of synthesized compounds were determined in open capillary tubes and are therefore uncorrected. The structures of compounds were established on the basis of elemental analysis and spectral data. The IR spectra were recorded in range of 4000-450 cm^{-1} using KBr pellets on a Perkin Elmer spectrophotometer. H NMR spectra were recorded on a Bruker DRX300MHz spectrophotometer using $\text{CDCl}_3/\text{DMSO-d}_6$ as solvent. Purity of synthesized compounds was checked by silica gel G plates of 2mm thickness using hexane, ethylacetate, water as solvent system and iodine chamber as developer.

Thin layer chromatography¹⁴

Evaluation of various mobile phases as solvents alone or in combination was done for each compound. The combinations introduced were hexane: ethyl acetate (1:1), benzene: ethyl acetate: water (6:4:1).

Development of chromatogram

Removed about 2 mm of the adsorbent from each edge of the plate to give a sharply defined edge. Applied about 2-5 μl volumes of a 10 mg^{-1} solution of the mixture and of reference substances in an organic solvent to the plate. Allowed the solvent to evaporate and transferred the plate to a previously prepared developing tank, this preparation was done about 30 minutes before insertion of the plate. Allowed the solvent to rise a distance of about 10 to 15 cm, removed the plate and allowed to dry.

Detection**Colouring agent**

Colouring agent used for the detection of the spot was done by using Iodine vapour. 1 gm of iodine crystals were placed on a chamber and was kept closed using a lid for saturation of the chamber with iodine vapour. The dried plates were kept over the chamber and were observed for spot in each plate. Colour of the spot was yellow.

UV lamp

The dried plates were kept in the UV chamber. The compounds which fluoresce to the given UV spots in the corresponding region Table 1.

MELTING POINT

The melting point is one of the physical data to evaluate the purity of the synthesized compounds. This was done by open capillary method. The melting range of the compounds define its purity Table 2.

Antibacterial activity^{15,16,17}**Preparation of inoculum**

Using a sterile inoculating loop or needle, touched four or five isolated colonies of the organism to be tested. Suspended the organism in 2 ml of sterile saline. Vortex the saline tube to create a smooth suspension. Adjusted the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy. Used this suspension within 15 minutes of preparation.

Inoculation of the MH plate

Dipped a sterile swab in to the inoculum tube. Rotated the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. Inoculated the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotated the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. Rim the plate with the swab to pick up any excess liquid. Discarded the swab into an appropriate container. Left the lid slightly ajar, allowed the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate was dried before proceeding to the next step.

Placement of the antibiotic disks

Placed the appropriate antimicrobial-impregnated disks on the surface of the agar, using forceps to dispense each antimicrobial disk one at a time. Placed the dispenser over the agar plate and firmly pressed the plunger once to dispense the disks onto the surface of the plate. Lifted the dispenser off the plate and using forceps sterilized by cleaning them with an alcohol pad touched each disk on the plate to ensure complete contact with the agar surface. This was done before replacing the Petri dish lid. Partially removed the lid of the Petri dish. Placed the disk on the plate over one of the dark spots on the template and

gently pressed the disk with the forceps to ensure complete contact with the agar surface. Replaced the lid to minimize exposure of the agar surface to room air. Continued to place one disk at a time onto the agar surface until all disks had been placed. Replaced the lid, inverted the plates, and placed them in a 35°C air incubator for 16 to 18 hours. DMSO was used as the control Table 3.

RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY

Staphylococcus aureus Gm (+) ve

The antibacterial activity of the test compounds were determined by Kirby-Bauer disk diffusion susceptibility test. All the test compounds showed comparable activity as that of the standard. Among the tested compound V, VI, VII showed maximum activity while compounds VIII showed better activity and compound I, II, III showed less activity.

Pseudomonas Gm (-) ve

The antibacterial activity of the test compounds were determined by Kirby-Bauer disk diffusion susceptibility test. All the test compounds showed comparable activity as that of the standard. Among the tested compound V and VIII showed maximum activity while compounds I, IV and VI showed better activity and compound II, III, VII showed minimum activity.

Table 1: The R_f values of the compounds were as follows

Sl. No.	Compound	R _f value
1	IM	0.79
2	I	0.78
3	II	0.74
4	III	0.75
5	IV	0.73
6	V	0.80
7	VI	0.68
8	VII	0.72
9	VIII	0.70
10	IX	0.67
11	X	0.78
12	XI	0.65
13	XII	0.76

Table 2: The melting points of the compounds were as follows

S. No.	Compound	Melting point (in °C)
1	IM	263 ^o C
2	I	160 ^o C
3	II	110 ^o C
4	III	237 ^o C
5	IV	90 ^o C
6	V	294 ^o C
7	VI	220 ^o C
8	VII	112 ^o C
9	VIII	216 ^o C
10	IX	250 ^o C
11	X	260 ^o C
12	XI	265 ^o C
13	XII	276 ^o C

Table 3: Anti-bacterial activity of synthesized compounds

COMPOUNDS	Zone of Inhibition against	
	Gram(+)veS.aureus (mm)	Gram(-)ve Pseudomonas (mm)
I	9	14
II	13	10
III	10	11
IV	15	13
V	18	15
VI	17	15
VII	17	9
VIII	16	16
Control	Resistant	Resistant
Standard	17	15

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