ejbps, 2019, Volume 6, Issue 8, 288-294. ***Research Article*** **SJIF Impact Factor 4.918**

EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 8
288-294
Year: 2019

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF MANNICH BASES OF COUMARINS

M. Sumakanth*, M. Sukanya and K. Sruthi

Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy.

***Corresponding Author: M. Sumakanth**
Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy.

Article Received on 05/06/2019 **Article Revised on 25/06/2019** **Article Accepted on 15/07/2019**

ABSTRACT

A series of Mannich bases of coumarin derivatives have been synthesized and were evaluated for their antibacterial activity. The starting material 7-Hydroxy-4-Methyl Coumarin which was prepared by Pechmann condensation was subjected to O-ethylation at 7-Position to yield 7-Ethoxy-4-Methyl Coumarin. The title compounds were inturn prepared by the mannich condensation of 7-Hydroxy/7-Ethoxy-4-Methyl Coumarin with a series of secondary amines. The structure of newly synthesized coumarin derivatives have been established on the basis of spectral (IR, ^{1}H NMR, Mass) data. The synthesized compounds were screened for anti bacterial activity against various gram positive and gram negative strains. The compounds demonstrated moderate to significant anti bacterial activity.

KEYWORDS: Mannich bases, Pechmann condensation, Coumarin, Anti bacterial activity.

INTRODUCTION

Coumarins[1,2] are naturally occurring oxygen containing heterocyclic compounds having diverse range of medicinal properties and hence they are used as lead compounds for designing new potent analogs. Coumarins form an important class of compounds of both which have both natural and synthetic origin.[4-6] Many compounds which contain the coumarin moiety exhibit useful and diverse pharmaceutical and biological activities[7-9], often depending on the substituents they bear in the parent benzopyran moiety and there has been a growing interest in their synthesis. Coumarins have long been recognized to exhibit anti-inflammatory, anti-oxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral and anti-carcinogenic activities. In addition to biological activities they are also used as additives to food and cosmetics and as optical brightening agents.

Mannich bases are beta-amino-ketones, formed by Mannich reaction[10-13], a nucleophilic addition reaction of a non-enolizable aldehyde with primary/secondary amine, forming resonance stabilized imine. Reactivity of these imines with carbanion produces the Mannich bases. The present study is envisaged towards the synthesis of a novel series of coumarin derivatives with Mannich bases and evaluated for their antibacterial activity.[13-16]

MATERIALS AND METHODS

Synthetic grade solvents and all the chemicals used were of synthetic grade obtained from SD fine chemicals, Merck and Sigma Aldrich. The solvents received from vendors were purified as per the prescribed methods and used for the synthesis. Reactions were monitored by using pre-coated Silica Gel TLC plates and the spots were visualised under UV light and in iodine chamber. The synthesized compounds were purified by recrystallization and purity was determined by measuring in Kofler hot stage melting point apparatus and are uncorrected. The IR spectrums were recorded on Shimadzu FTIR spectrophotometer by using 1% KBR discs. Proton NMR was recorded on Bruker Avance II 400 MHz NMR spectrophotometer with TMS as internal standard mass spectrum was recorded using Agilent 1100.

RESULTS AND DISCUSSION

Synthesis

The synthesis of Mannich bases of Coumarin derivatives 5a-d & 7a-d was achieved from the synthetic route depicted in **figure 3**. The 7-Hydroxy-4-Methyl Coumarin have been synthesized by the reaction of resorcinol(1) with ethylacetoacetate(2). The reaction progress was monitored by TLC and the formation of the compounds was confirmed by melting point and spectral data. The IR spectra of the compound showed characteristic peak at 1743cm^{-1} which corresponds to C=O δ-lactone stretching, 3529 cm^{-1} of O-H stretching and at 1265cm^{-1} which corresponds to C=O stretching and at 2960cm^{-1} of Aliphatic C-H stretching), 1481, 1450, 1431 cm^{-1}of Aromatic C=C stretching respectively. ^{1}H NMR (DMSO) δ 2.3 (s,CH_3), δ 9.8 (s ,OH), δ 6.3-7 (m, Ar-H).), the characteristic singlet of CH_3 proton at δ 2.3 and another characteristic –OH singlet at δ 9.8 were observed in proton NMR spectra.

The obtained 7-Hydroxy-4-Methyl Coumarin(4) were inturn reacted with ethyl iodide and potassium carbonate in acetone using ethanol under reflux conditions to yield 7-Ethoxy-4-Methyl Coumarin(6). The IR spectra of the compound showed the characteristic absorption peak at 2819cm^{-1} corresponds to C-O stretching indicates the substitution at 7th position which confirms the formation of compound. In the next step each of the 7-Hydroxy/7-Ethoxy-4-Methyl Coumarin was subjected to Mannich reaction with a series of 2^0 amines like dimethyl amine, diethyl amine, piperidine and morpholine respectively. The reaction conditions, have been found to yield a single product in each case. All the compounds (5a-5d, 7a-7d) were obtained in good yields and were characterized by melting point, proton NMR, FTIR and mass spectral data. The formation of title compound (7d) was confirmed by FTIR with the characteristic C-H stretching at (2941.44cm^{-1}), O-H stretching (3431.36cm^{-1}), C-N stretching(1327.03cm^{-1}), δ-lactone (1743.65cm^{-1}) which indicates (dimethyl amino)methyl substitution at 8-position has occurred. In ^{1}HNMR, the characteristic singlet of CH_3 proton (position 4) at δ 2.43 and another characteristic –OH singlet (position 7)at δ 8.03 and characteristic doublet (dimethyl) at δ 2.27, 2.27 were observed in proton NMR spectra and mass peak is observed at m/z of 232 .

Anti-bacterial activity

The synthesized compounds of Mannich bases of coumarin derivatives were evaluated for the antibacterial activity against *E.coli* (NCIM 2068) *P.aeruginosa(*NCIM 2862*)*, *S.aureus(*NCIM 2079*)*, *B.subtilis(*NCIM 2921*)* bacterial strains employing disc diffusion method using Gentamycin as the standard (10µg/ml).

Filter paper discs impregnated with test and standard solutions were placed appropriately on the prepared petriplates under aseptic conditions. The plates were then kept at room temperature for the diffusion of solution into the media, further the plates were incubated at 37±1ºC for one day (24hr). After the completion of the incubation period the zone of inhibition of the bacterial growth was measured. Petri plates were also maintained for simultaneously for both positive controls and negative controls. Each of the tests was carried in triplicates and the standard deviation was calculated. The results of antibacterial activity of the title compounds diameter of the zone of inhibition are presented in the table.

The synthesized compounds were evaluated for their antibacterial activity, from the results it was concluded that the compounds showed higher sensitivity towards Gram positive bacteria i.e., *S.aureus (*NCIM 2079*)* and *B.subtilis (*NCIM 2921*)* than Gram negative bacteria *E.coli* (NCIM 2068) *P.aeruginosa (*NCIM 2862*)* respectively. Compounds 7c and 7d exhibited remarkable inhibition on the growth of all the bacterial strains respectively. Apparently, all the remaining compounds demonstrated moderate anti bacterial activity on *S.aureus (*NCIM 2079*)* and *B.subtilis (*NCIM 2921*)* than Gram negative bacteria *E.coli* (NCIM 2068) and comparatively weaker inhibition on *P.aeruginosa (*NCIM 2862*)*. It was evident from the above results that the mannich bases of coumarin derivatives could be used as better alternatives for the treatment of bacterial diseases.

I. Anti bacterial Activity of title compounds against *Bacillus subtilis and Staphylococcus aureus* at various concentrations

Compound	***Bacillus subtilis***					***Staphylococcus aureus***				
Conc(µg/ml)	**10**	**25**	**50**	**75**	**100**	**10**	**25**	**50**	**75**	**100**
5a	0.9	2.1	3.4	4.1	5.4	1.1	2.1	3.1	6.1	7.2
5b	0.8	2.3	3.2	4.4	6.3	2	3.4	4	5.5	7.4
5c	0.7	2.4	3.5	5.3	7.2	1.8	2.3	3.2	5.4	7.5
5d	1.9	2.1	3.6	4.2	8.1	1.4	2.5	4.5	6.1	7.9
7a	0.6	1.9	3.1	4.5	5.3	1.3	2.7	4.1	4.8	7.4
7b	0.7	2.2	4	5.1	8.4	2.5	3.5	4.2	5.9	5.9
7c	0.8	2.3	8.2	11	13	3.1	5.1	**7.1**	**7.6**	**11**
7d	1	2.1	3.4	4.3	6.2	1.3	6.3	**7.2**	**8.3**	**10.4**
Ciprofloxacin (10µg/ml)	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6

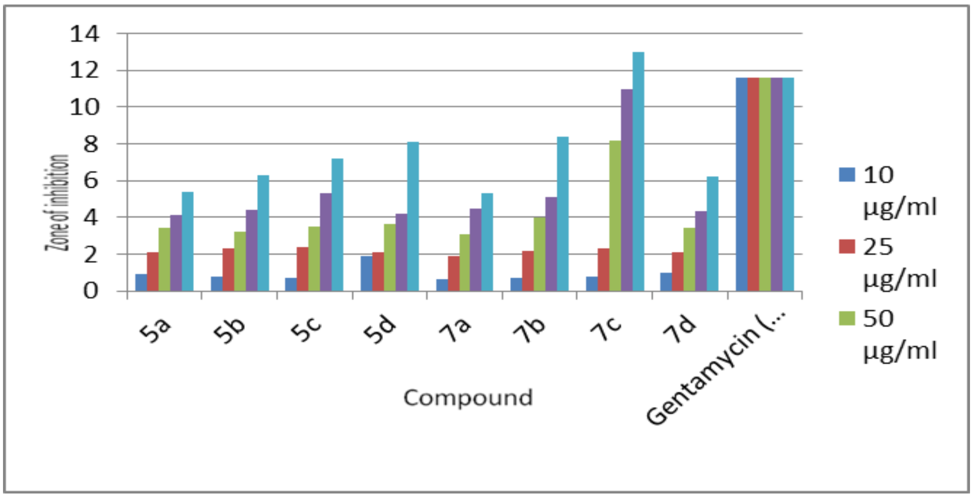


Fig. 1: Graphical Representation of Anti bacterial Activity of title compounds against *Bacillus subtilis* at various concentrations.

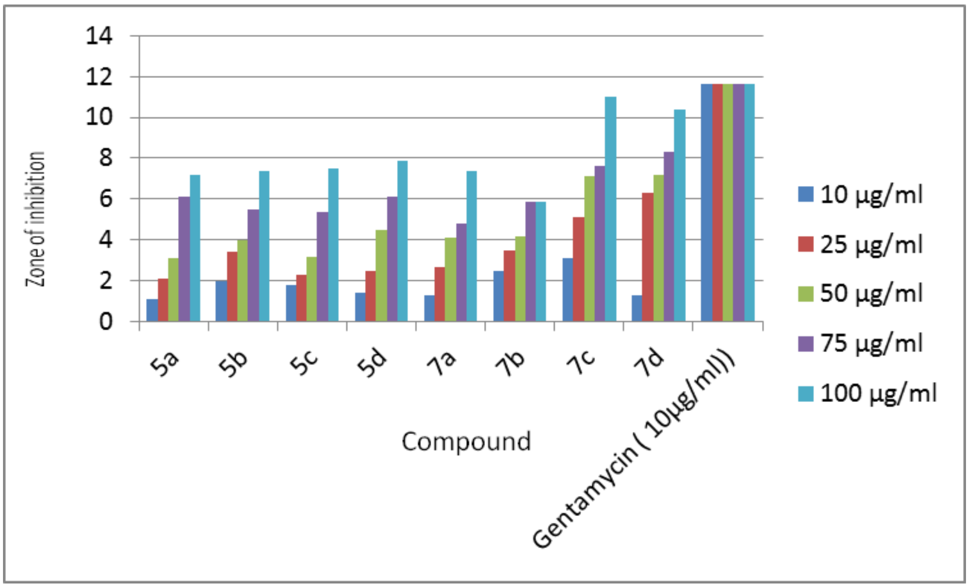


Fig. 2: Graphical Representation of Anti bacterial Activity of title compounds against *Staphylococcus aureus* at various concentrations.

II. Anti bacterial Activity of title compounds against *Pseudomonas aeruginosa and Escherichia coli* at various concentrations

Compound	***Pseudomonas aeruginosa***					***Escherichia coli***				
Conc(µg/ml)	10	25	50	75	100	10	25	50	75	100
5a	-	-	-	-	-	2.1	3.1	4.6	5.1	7.1
5b	-	-	-	-	-	1.9	4	5.3	5.2	**8.9**
5c	-	-	-	-	-	2.4	3.3	4.1	4.4	**8.1**
5d	-	-	-	-	-	3.2	4.1	5.2	4.2	5.3
7a	-	-	-	-	-	2.1	3.7	4.4	4.6	6.1
7b	-	-	-	-	-	3.1	4.2	5.1	4.3	5.2
7c	4.5	6.2	8.1	7.2	9.3	1.9	3.2	4.5	5.4	**8.3**
7d	-	-	-	-	-	4	5.1	5.6	6.1	7.4
Ciprofloxacin (10µg/ml)	11.5	11.5	11.5	11.5	11.5	11.7	11.7	11.7	11.7	11.7

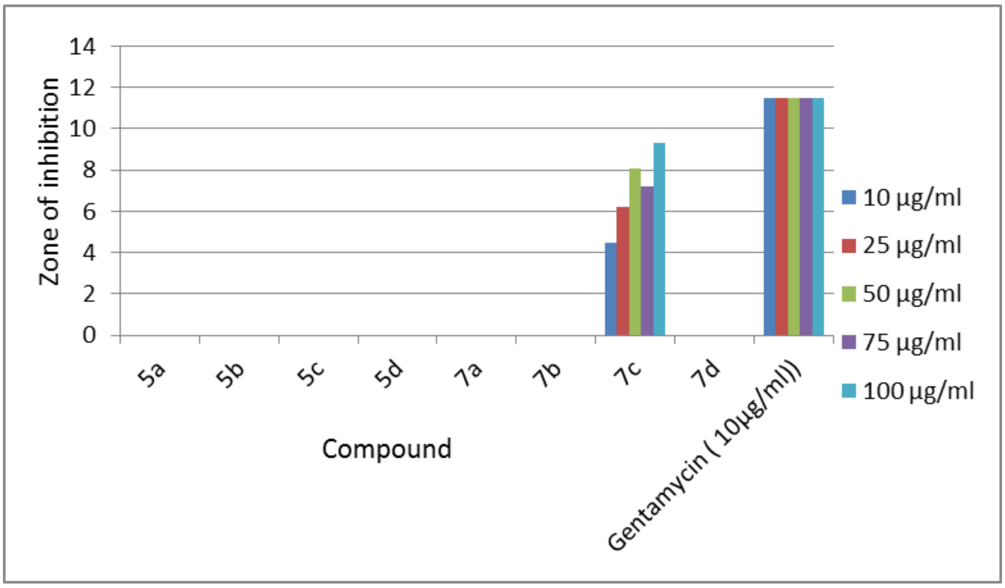


Fig. 3: Graphical Representation of Anti bacterial Activity of title compounds against *Pseudomonas aeruginosa* at various concentrations.

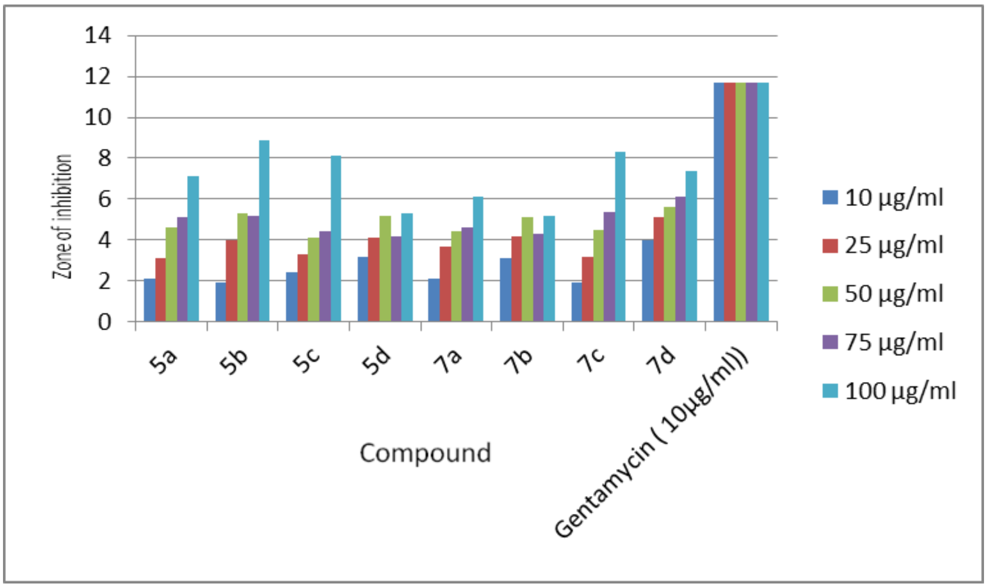


Fig. 4: Graphical Representation of Anti bacterial Activity of title compounds against *Escherichia coli* at various concentrations.

EXPERIMENTAL

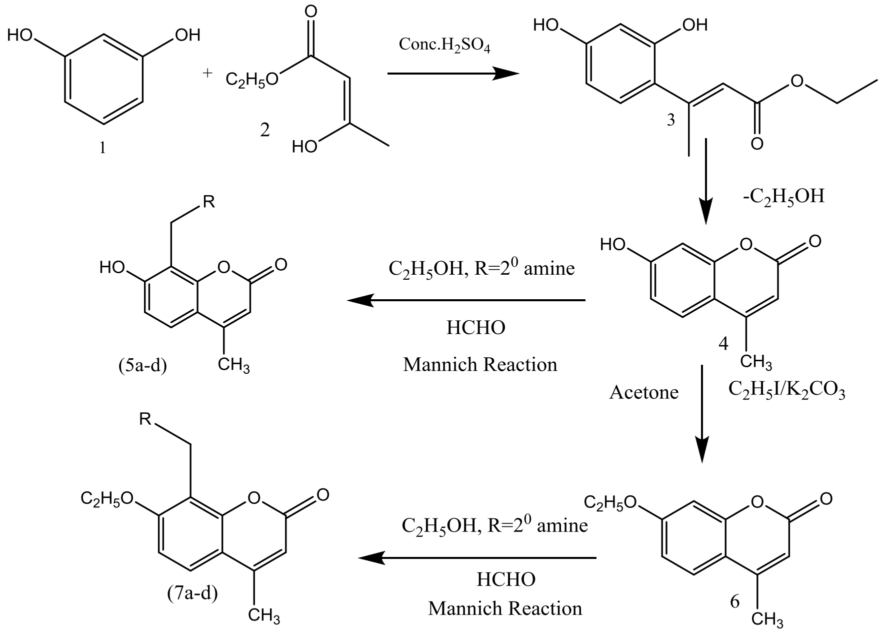
Synthesis of Mannich bases of Coumarin Derivatives

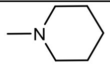
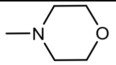
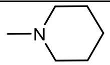
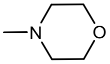
SYNTHESIS OF 7-Hydroxy-4-Methyl Coumarin(4): Concentrated Sulphuric acid (50ml) was taken in a 3-necked round bottomed flask (250ml) and cooled to 10^0C. Powdered resorcinol (5g,0.04M) dissolved in freshly distilled ethyl acetoacetate (6.8ml,0.05M) was taken in a dropping funnel. The mixture of resorcinol and ethyl acetoacetate was added dropwise to cold Conc. Sulphuric acid solution with constant stirring. The temperature was maintained always below 10^0C. After adding all the portions of the resorcinol solution and ethyl acetoacetate mixture, the solution was kept aside for cooling 24hrs, without further cooling. Then the reaction mixture was poured into crushed ice (150gms). The precipitate obtained was filtered and dried. The dried product was dissolved in NaOH Solution(10%) then acidified with dilute Sulphuric acid till the solution was acidic to litmus. The solution was kept aside for 10 minutes. The formed precipitate was washed with water and recrystallized from the ethanol. Yield-50.6%, M.P-180-190^0c.

SYNTHESIS OF MANNICH BASES OF COUMARIN DERIVATIVES (5a-d & 7a-d): In a 250ml three necked round bottomed flask attached to a reflux condenser are placed 3g (0.5 mole) of 7-Hydroxy-4-Methyl Coumarin (7ml, 0.65ml) of dimethylamine and 1g (0.22 mole) of paraformaldehyde. After the addition of 0.04ml of concentrated Hydrochloride in 8ml of 95% ethanol, the mixture is refluxed on a waterbath for 2 hours. The yellowish solution is filtered. If it is not clear, it is transferred to Erlenmeyer flask. Subsequently the solvents were removed by distillation and the solid mass obtained was washed with water. It was dried in vacuum and recrystallised from methanol. Yield-2gms, 78-82% and melts at 138-141^{0}C.

Further the derivatives were obtained by mannich reaction of 7-Hydroxy-4-Methyl Coumarin with diethyl amine, piperidine, morpholine, dimethyl amine, diethyl amine to yield mannich bases of 7-Hydroxy-4-Methyl Coumarin.

Scheme For The Synthesis Of Mannich Bases Of Coumarin Derivatives Is Outlined Below



Compound	**R**
5a	**-N(CH$_3$)$_2$**
5b	**-N(C$_2$H$_5$)$_2$**
5c	
5d	
7a	**-N(CH$_3$)$_2$**
7b	**-N(C$_2$H$_5$)$_2$**
7c	
7d	

5a: 8-(Dimethyl amino)methyl-7-Hydroxy-4-Methyl Coumarin: Yield: 2.59g(84%), M.P(˚C): 126-128, IR(KBr) (cm-1):2941.44(-CH$_2$- stretching), 3431.36 (O-H stretching), 802,823,850(Aromatic C-H stretching), 1431,1500,1543(Aromatic C=Cstretching. ^{1}H NMR: δ 2.41 (3H, s), 2.44 (6H, s), 3.97 (2H, s), 5.87 (1H, s), 6.59 (1H, d, *J* = 8.4 Hz), 7.29 (1H, d, *J* = 8.4 Hz).

5b: 8-(Diethyl amino)methyl-7-Hydroxy-4-Methyl Coumarin: Yield: 2.2g(82%), M.P(˚C): 124-126, IR(KBr) (cm-1):2941.44(-CH$_2$- stretching), 3529.73 (O-H stretching), 1265.3(C-O stretching), 1327.03(3^0 N stretching), 1431,1460,1543(Aromatic C=Cstretching). ^{1}H NMR: δ 0.99 (6H, t, *J* = 7.2 Hz), 2.41 (3H, s), 2.67 (4H, q, *J* = 7.2 Hz), 3.98 (2H, s), 5.88 (1H, s), 6.59 (1H, d, *J* = 8.4 Hz), 7.29 (1H, d, *J* = 8.4 Hz),m/z ratio:262.

5c: 7-Hydroxy-4-Methyl-8-(Piperidin-1-yl) Coumarin: Yield: 2.1g (81%), M.P(˚C): 137-139, IR(KBr) (cm-1):2933.73(-CH$_2$- stretching), 3527.8 (O-H stretching), 1265.3(C-O stretching), 1344.38(3^0 N stretching), 1734 (C-O δ-lactone) 1384,1442,1447 (Aromatic C=C stretching). ^{1}H NMR: δ 1.47 (2H, dtt, *J* = 10.9, 10.2, 3.3 Hz), 1.73 (4H, ddddd, *J* = 10.9, 3.3, 2.9, 2.7, 2.4 Hz), 2.41 (3H, s), 2.52 (4H, ddd, *J* = 13.0, 10.3, 2.7 Hz), 3.90 (2H, s), 5.88 (1H, s), 6.59 (1H, d, *J* = 8.4 Hz), 7.29 (1H, d, *J* = 8.4 Hz), m/z ratio:274.

5d: 7-Hydroxy-4-Methyl-8-(Morpholino methyl) Coumarin: Yield: 2.5g (84%), M.P(˚C): 136-138, IR(KBr) (cm-1):2920.23(-CH$_2$- stretching), 3412.08 (O-H stretching), 1220.94(C-O stretching), 1367.53(3^0 N stretching), 1718.58 (C-O δ-lactone) 1527,1597,1571(Aromatic C=C stretching). ^{1}H NMR: δ 2.41-2.54 (7H, 2.48 (ddd, *J* = 11.9, 10.2, 2.5 Hz), 2.41

(s)), 3.60 (4H, ddd, *J* = 12.2, 3.1, 2.5 Hz), 3.97 (2H, s), 5.88 (1H, s), 6.59 (1H, d, *J* = 8.4 Hz), 7.29 (1H, d, *J* = 8.4 Hz).

7a: 8-(Dimethyl amino)methyl-7-Ethoxy-4-Methyl Coumarin: Yield: 2.4g (82%), M.P(˚C): 124-126, IR(KBr)(cm-1):2941.44**(**-CH_2- stretching), 1265.3(C-O stretching), 1327.53(3^0 N stretching),1743.65 (C-O δ-lactone) 1431,1460,1500(Aromatic C=C stretching). ^{1}H NMR: δ 1.30 (3H, t, *J* = 7.0 Hz), 2.41 (3H, s), 2.44 (6H, s), 3.95 (2H, s), 4.24 (2H, q, *J* = 7.0 Hz), 5.88 (1H, s), 6.64 (1H, d, *J* = 8.4 Hz), 7.28 (1H, d, *J* = 8.4 Hz).

7b: 8-(Diethyl amino)methyl-7-Ethoxy-4-Methyl Coumarin: Yield :2.2g (82%), M.P(˚C): 124-126, IR(KBr)(cm-1): 2961.44**(**-CH_2- stretching), 1275.3(C-O stretching), 1357.53(3^0 N stretching),1743.65 (C-O δ-lactone) 1451,1470,1510(Aromatic C=C stretching). ^{1}H NMR: δ 0.99 (6H, t, *J* = 7.2 Hz), 1.30 (3H, t, *J* = 7.0 Hz), 2.41 (3H, s), 2.71 (4H, q, *J* = 7.2 Hz), 3.96 (2H, s), 4.24 (2H, q, *J* = 7.0 Hz), 5.88 (1H, s), 6.65 (1H, d, *J* = 8.4 Hz), 7.28 (1H, d, *J* = 8.4 Hz), m/z ratio:290.

7c: 7-Ethoxy-4-Methyl-8-(Piperidin-1-yl)Coumarin: Yield: 2.1g (81%), M.P(˚C): 132-134, IR(KBr)(cm-1):2933.73**(**-CH_2- stretching), 1220.94(C-O stretching), 1344.38(3^0 N stretching),1744.06 (C-O δ-lactone) 1442,1477,1529 (Aromatic C=C stretching). ^{1}H NMR: δ 1.30 (3H, t, *J* = 7.0 Hz), 1.48 (2H, dtt, *J* = 10.9, 10.2, 3.3 Hz), 1.72 (4H, ddddd, *J* = 10.9, 3.3, 2.9, 2.7, 2.4 Hz), 2.41 (3H, s), 2.52 (4H, ddd, *J* = 13.1, 10.3, 2.7 Hz), 4.01 (2H, s), 4.26 (2H, q, *J* = 7.0 Hz), 5.88 (1H, s), 6.65 (1H, d, *J* = 8.4 Hz), 7.28 (1H, d, *J* = 8.4 Hz), m/z ratio:302.

7d: 7-Ethoxy-4-Methyl-8-(Morpholino methyl) Coumarin: Yield :2.1g (81%),M.P(˚C): 132-134, IR(KBr)(cm-1):2960.73**(**-CH_2- stretching), 1265.30(C-O stretching), 1325.10(3^0 N stretching),1743.65 (C-O δ-lactone) 1431,1481,1388(Aromatic C=C stretching). ^{1}H NMR: δ 1.30 (3H, t, *J* = 7.0 Hz), 2.41-2.54 (7H, 2.48 (ddd, *J* = 12.1, 10.2, 2.5 Hz), 2.41 (s)), 3.61 (4H, ddd, *J* = 12.3, 3.1, 2.5 Hz), 4.07 (2H, s), 4.24 (2H, q, *J* = 7.0 Hz), 5.88 (1H, s), 6.65 (1H, d, *J* = 8.4 Hz), 7.28 (1H, d, *J* = 8.4 Hz).

CONCLUSION

In the present investigation a new series of Mannich bases of 7-Hydroxy/Ethyl-4-Methyl Coumarin derivatives were synthesized and these compounds were characterized by FTIR, Mass and 1H NMR spectral data. All the title compounds were evaluated for the antibacterial activity against *E.coli* (NCIM 2068) *P.aeruginosa(*NCIM 2862*)*, *S.aureus(*NCIM 2079*)*, *B.subtilis(*NCIM 2921). All the compounds tested were found to be moderately active on the tested bacterial strains particularly on gram positive bacteria and these compounds could be considered as potential antibacterial agents.

REFERENCES

1. Musa, M.A.; Badisa, V.L.; Latinwo, L.M.; Cooperwood, J.; Sinclair, A.; Abdullah, A. Cytotoxic activity of new acetoxycoumarin derivatives in cancer cell lines. Anticancer Res., 2011; 31: 2017–2022.
2. Borah, P.; Naidu, P.S.; Bhuyan, P.J. Synthesis of some tetrazole fused pyrido[2,3-c]coumarin derivatives from a one-pot three-component reaction via intramolecular 1,3-dipolar cycloaddition reaction of azide to nitriles, Tetrahedron Lett., 2012; 53: 5034–5037.
3. El-Ansary, S.L.; Abbas, S.E.; Mikhael, A.N.; El-Banna, H.A. Synthesis and biological activity of some new coumarins. Egypt. J. Pharm. Sci., 1992; 33: 639–650. Molecules, 2016; 21: 249 18-20.
4. Manfredini, S.; Daniele, S.; Ferroni, R.; Bazzanini, R.; Vertuani, S.; Hatse, S.; Balzarini, J.; de Clercq, E.retinoic acid conjugates as potential antitumor agents: synthesis and biological activity of conjugates with Ara-A, Ara-C, 3(2H)-furanone, and aniline mustard moieties. J. Med. Chem., 1997; 40: 3851–3857.
5. Wattenberg, L.W.; Lam, K.T.; Fladmoe, A.V. Inhibition of chemical carcinogen-induced neoplasia by coumarins and *a*-angelicalactone. Cancer Res., 1979; 39: 1651–1654.
6. Kashman, Y.; Gustafson, K.R.; fuller, R.W.; Cardellina, J.H.; McMahon, J.B.; Currens, M.J.; Buckheit, R.W.; Hughes, S.H.; Craqq, G.M.; Boyd, M.R. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, Calophyllum lanigerum. J. Med. Chem., 1992; 35: 2735–2743.
7. Mckee, T.C.; Fuller, R.W.; Covington, C.D.; Cardellina, J.H.; Gulakowski, R.J.; Krepps, B.L.; McMahon, J.B.; Boyd, M.R. New pyranocoumarins isolated from Calophyllum lanigerum and Calophyllum teysmannii, J. Nat. Prod., 1996; 59: 754–758.
8. Anjum, N.F.; Aleem, A.; Nayeem, N.; Asdaq, S.M. Synthesis and antibacterial activity of substituted, 2-phenyl-4-chromones. Der Pharma Chem., 2011; 3: 56–62.
9. Vijayan KK, Nidhin P, Ranjith CA. Green approach for the synthesis of Coumarin Derivative by microwave assisted method. Pharmazie, 1995; 12: 2-10.
10. Kontogirogis C, Hadjiipavlou-Litina D. Biological evaluation of several coumarin derivatives designed as possible antioxidant agents. J Enzyme Inhib Med Chem., 2003; 8: 63.
11. Khode S, Maddi V, Aragade P, Palkar M, Ronad PK, Mamledesai S, Thippeswamy AHM, Satyanarayana D. Synthesis and pharmacological evaluation of a novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents. Eur. J. Med. Chem., 2009; 44(4): 1682-1688.

12. Kontogiorgis CA, Hadjipavlou-Litina, DJ. Synthesis and Antiinflammatory Activity of Coumarin Derivatives. J. Med. Chem., 2005; 48(20): 6400-6408.
13. Hoult J, Payd, M. Pharmological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen. Pharmacol, 1996; 27: 713-722.
14. Matern U, Luer P, Kreusch D. Biosynthesis of coumarins. In Comprehensive Natural Products Chemistry. Amsterdam: Elsevier, 1999; 623-637.
15. Ramanitrahasimbola D, Rakotondramanana DA, Rasoanaivo P, Randriantsoa AS, Ratsimamanga PG, Galeffi C, Nicoletti M. Bronchodilator activity of Phymatodes scolopendria (Burm.) Ching and its bioactive constituent. J. Ethnopharmac, 2005; 102(3): 400-407.
16. Aziz B, Islam K, Skender D. Antibacterial Activity of Coumarine Derivatives Synthesized from 4,7-Dihydroxy-2-oxo-2H-chromene-3-carbaldehyde and Comparison with Standard Drug. Int. J. Pharm. Bio. Sci., 2001; 45: 501-508.