Knowledge Consortium of Gujarat
Department of Higher Education - Government of Gujarat
Journal of Science
ISSN: 2320 - 0006

Continuous issue-3 | September - October 2013

Studies on Antimicrobial Activity of Formazans containing Coumarinyl Moiety

Abstract

Compounds bearing Formazan moiety are endowed with a variety of pharmacological activities such as insecticidal(1), bactericidal(2), antiviral(3), antiinflamatory(4); Antiparkinsonian(5) acivity. Again the Coumarinyl moiety have been reported to have excellent physiological activity few of which are anthelmintic, antiallergic, antiartheritic, anticancerous, antimalerial antinaphylactic,antiproliferative, and more large number of activities. Looking over to these properties it was contemplated to synthesis some new Formazans having Coumarinyl moiety which may enhance the biological activity with least side effect. The structures have been characterized by the elemental analysis and the spectral data. The compounds were screened for their antimicrobial activity using different strains of Bacteria's and Fungi.

Keywords:(1) Coumarin Schiff's base (2) Formazans

[1] Introduction :

Benzopyrones forms a fascinating group of the compounds occurring widely both in free; combined states. Benzo-α pyrones so called coumarin is a mile stone in a path of natural; chemistry due to its varied biochemical; analytical applications[6]. Due to its varied industrial use in perfumery, backery, beverages, soap, tobacco, rubber; plastic industries, a considerable amount of work has been on coumarins; has been reviewed by a number of workers[7,8]. Coumarin derivatives are reported to have an excellent biological activity such as anthelmintic[9], antiallergic,antiarthritic[10], antibacterial[11], anticancerous[12], anticoagulant[13], antifungal[14], antiinflamatory[15], antimalarial[16],antinaphylactic[10], antiproliferative[17], antispasmodic[18], hypnotic[19], hypolipidimic[20], hypotensive[21], insecticidal[22], antifertility[23], potential nervous system depressant, sedative[24]. It would enhance the therapeutic activity, if the coumarinyl moiety is joined with the formazan moiety. Several methods are employed for its synthesis[31,32,33]. Taking in view the pharmaceutical utility of the formazans we undertook the preparation of its derivatives as under.

[2] Experiment : al

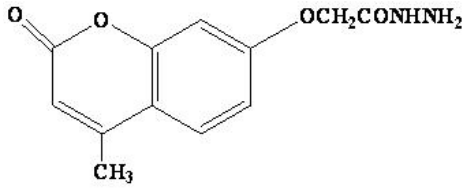
All the melting points are taken in an open capillary tube and all uncorrected I.R spectra (KBr) were recorded on Perkin Elmer spectrometer and 1H NMR spectrometer at 300MHz. the purity of compound was checked by TLC using Silica gel-G.

(I) Preparation of the Schiff base:-

(A) Preparation of 4-methyl-7-hydrazino-carbonylmethoxycoumarin[27]

The 7-Hydroxy-4-methyl coumarin was esterified stirring for 12 hrs. with ethylchloroacetate in acetone and refluxed. The ester formed was then taken in rectified spirit to which hydrazine hydrate was added and further refluxed for 8 Hrs., then after it is cooled and poured in ice to give crystalline product with (m.p. -115ºC).yield 75%

(Found C–58.06%, H-4.83% and N-11.29%; Calculated C–58.10%, H-4.78% and N-11.33%) For $C_{12}H_{12}N_2O_4$



(B) Preparation of Schiff's Base
[4-methyl-7-(substituted benzylhydrazinocarbonylmethoxy) coumarin][27]

A mixture of hydrazine (0.01M, 2.48 gm) was dissolved in alcohol then p-Anisaldehyde
(0.01 M, 1.36 gm) was added to it, refluxed for four hours. The reaction mixture was cooled and the product was isolated as well as crystallized in DMF to give shinning white crystal (m.p 248ºC) Yield 75%, (Found C–65.52%, H-4.9% and N-7.62%; Calculated C–65.57% , H-4.9% and N-7.65%) For $C_{20}H_{18}N_2O_5$.

Similarly other Schiff bases were prepared.The physical constants are recorded in Table no-1 :

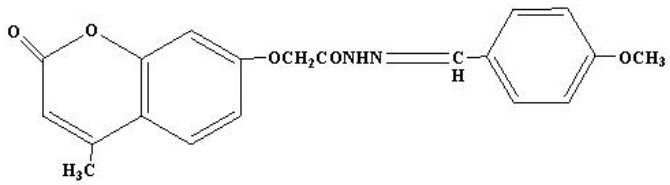


TABLE NO. 1 :

[A] PHYSICAL CONSTANT OF 4-METHYL-7-[(4'-METHOXYBENZYLHYDRAZINOCARBONYLMETHOXY] COUMARIN

Sr. No.	R.	Mol. Form.	m.p ºC	percentage %

				Yield	N Calc.	N found
1	Phenyl	$C_{19}H_{16}N_2O_4I$	254	70	8.30	8.28
2	3-Aminophenyl	$C_{19}H_{17}N_3O_4$	256	65	11.97	11.94
3	4-Aminophenyl	$C_{19}H_{17}N_3O_4$	225	65	11.97	11.95
4	5-bromo-4-hydroxy -3-methoxyphenyl	$C_{19}H_{15}N_2O_4Br$	250	70	6.07	6.06
5	2-chlorophenyl	$C_{19}H_{15}N_2O_4Cl$	263	70	7.56	7.54
6	5-chlorophenyl	$C_{19}H_{15}N_2O_4Cl$	218	70	7.56	7.52
7	3,4-dibromo-2-hyroxy Phenyl	$C_{19}H_{14}N_2O_5Br_2$	268	65	5.50	5.52
8	3,4-dichlorophenyl	$C_{19}H_{14}N_2O_4C_{12}$	265	65	6.93	6.92
9	3,4-dimethoxyphenyl	$C_{21}H_{20}N_2O_6$	270	65	7.07	7.08
10	3,4-dimethoxy-5-nitrophenyl	$C_{21}H_{19}N_3O_8$	175	75	9.52	9.52
11	4-Methoxyphenyl	$C_{20}H_{18}N_2O_5$	248	75	7.65	7.62

SPECTRAL DATA
[B] N.M.R SPECTRAL DATA.

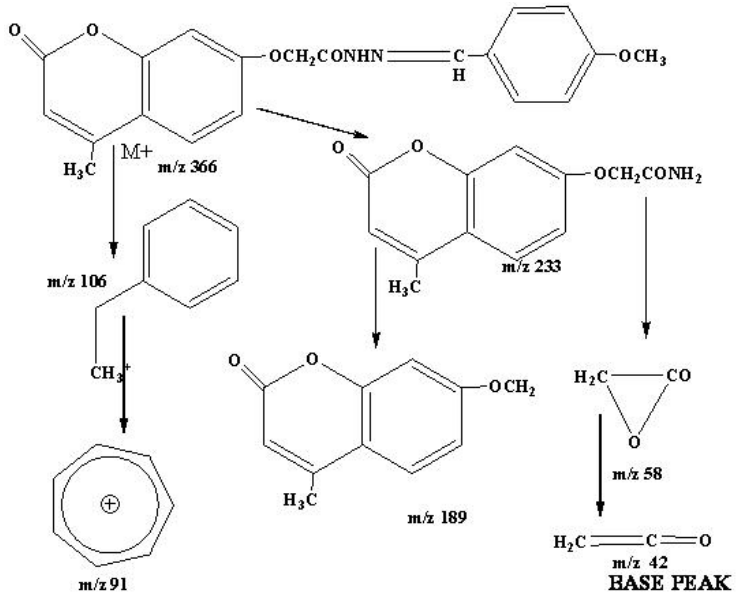
Single.No.	δ p.p.m	No.of protons	Multiplicity	inference
1	2.35	3H	singlet	$-CH_3$
2	3.75	3H	singlet	$-OCH_3$
3	4.60	2H	singlet	$-OCH_2-$
4	6.80	1H	singlet	-N=C<u>H</u>-Ar
5	6.9	1H	singlet	-C<u>H</u>-coumarin
6	7.5-7.9	8H	Multiplet	Aromatic H
7	8.1	1H	singlet	-CO-N<u>H</u>-N=

[C] IR SPECTRAL STUDY (SHIMADZU-2245)

TYPE	Vibration Mode	Freq in cm^{-1}		Reference
		Obs.	Reported	
Alkane $-CH_3$	-C-H str.(asym)	2950	2975-2950	28-29
$-CH_2$	-C-H str. (sym)	2855	2880-2860	
	-C-H str.(asym)	2935	2940-2915	
	-C-H str. (sym)	2875	2890-2845	
Aromatic	-C-H str.	3050	3080-3030	

(1-4-disubst.)	-C=C- str.	1620	1612-1600	
		1580	1585-1573	
		1500	1520-1480	
		1405	1417-1401	
Amide -CO-NH-N-	-N-H. str. (asym.)	3455	3550-3250	
	-N-H. str. (sym.)	3310	3450-3250	
	-N-H. def,	1550	1650-1580	
	-C-N. str.	1120	1220-1020	
	-C=O str.	1690	1680-1630	
	Schiffbase linkage	1630	1690-1580	
$-CH_3$	-C-O-C- (asym.)	1275	1275-1200	
	-C-O-C (sym.)	1050	1075-1020	
Coumarin moiety	-C=O	1725	1725-1730	
		1275	1275-1200	

[D] MASS SPECTRA



II–PREPARATION OF: -4-METHYL-7-[α-(p-CHLOROPHENYLAZO)p-METHOXY BENZALHYDRAZINO CARBONYLMETHOXY]COUMARIN.

p-chloroaniline (2.5gm0.02M) in glacial acetic acid (2 ml) and HCl (1.5ml) was diazotized with $NaNO_2$ (2gm in 2ml water) in cold 0-5,ºC. The resultant diazonium chloride solution was added to the schiff's base (3.66gms 0.01M) in pyridine at low temperature (0-5ºC) and the reaction mixture was stirred gently and left overnight at ambient temperature. Thereafter it was poured into cold water. The product was isolated and crystallized from chloroform m.p. 130ºC decomposes yield 40% (C-61.82%; H-4.12 %; N-11.10% and Calculated C –61.84% , H-4.16 % and N-11.10%) for $C_{26}H_{21}N_4O_5Cl$

The formazans were characterized by elemental analysis as well as supported by its various spectroscopic data as shown in Table-II.

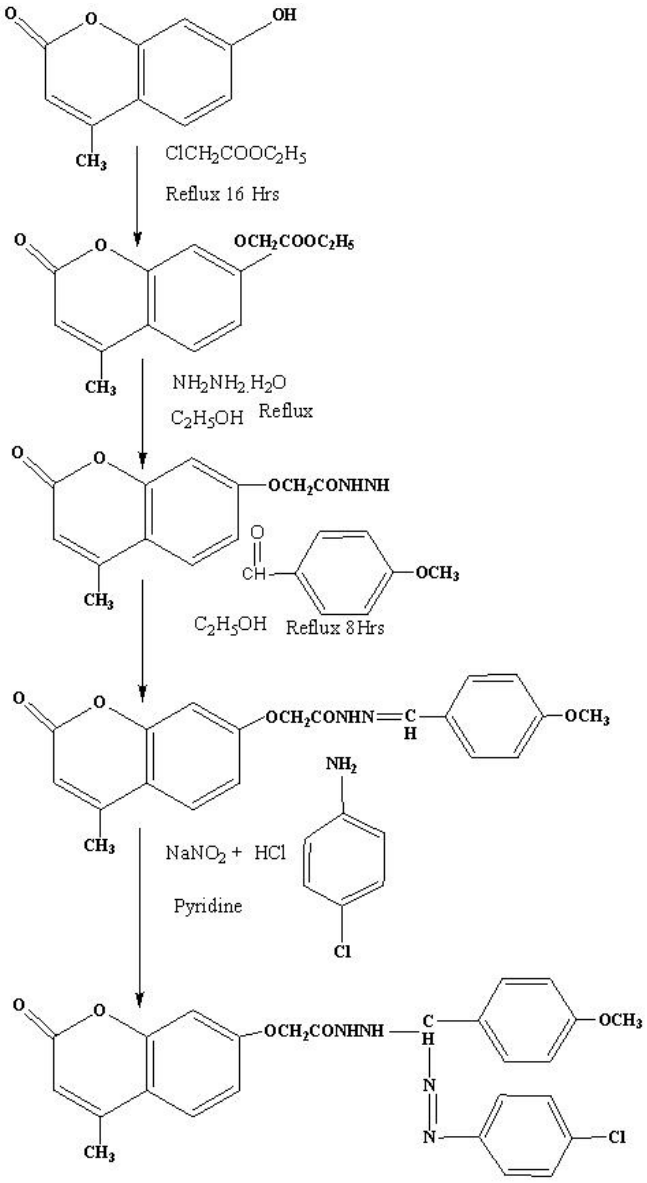


TABLE No. – 2
[A] PHYSICAL CONSTANTS

4-METHYL-7-[□-(P-CHLOROPHENYLAZO)SUBSTITUTEDBENZALHYDRAZINO CARBONYLMETHOXY]COUMARIN.

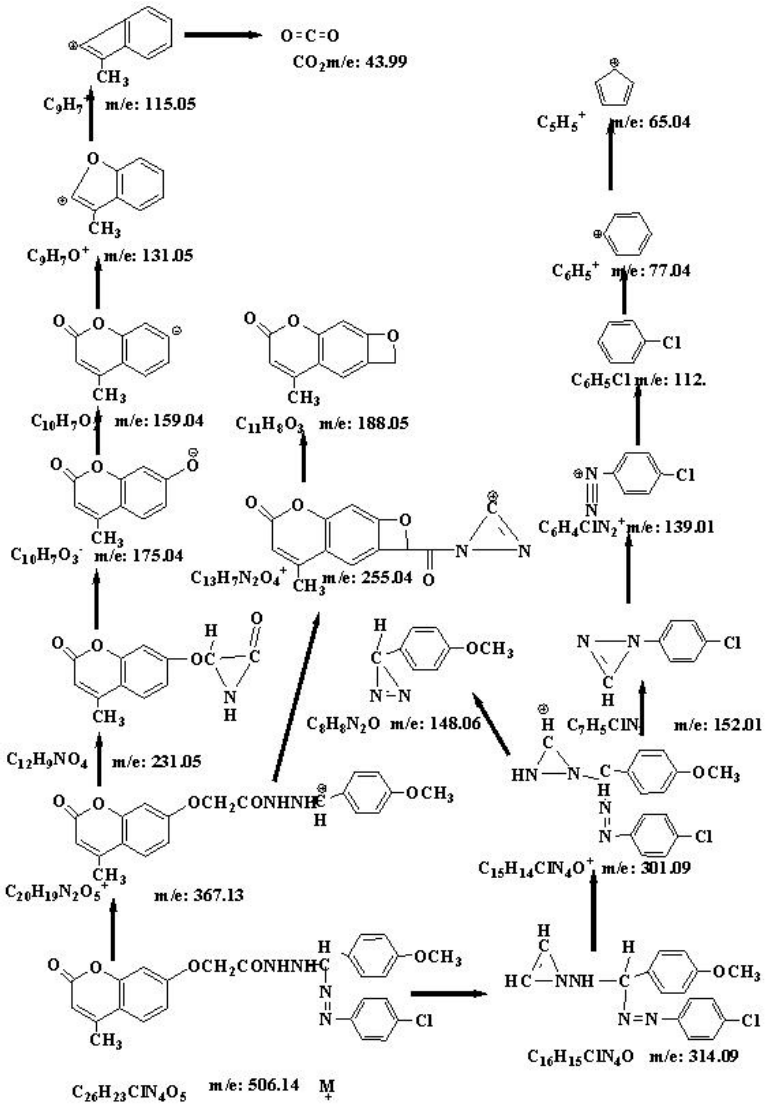
Sr. No.	R.	Mol. Form.	m.p °C	Percentage %		
				Yield	N Calc.	N found
1	Phenyl	$C_{25}H_{19}N_4O_4Cl$	155	50	11.80	11.88
2	3-Aminophenyl	$C_{25}H_{20}N_5O_4Cl$	129	55	14.30	14.20
3	4-Aminophenyl	$C_{25}H_{20}N_5O_4Cl$	120	55	14.30	14.31
4	5-bromo-4-hydroxy -3-methoxyphenyl	$C_{26}H_{20}N_4O_6ClBr$	160	55	9.34	9.25
5	2-chlorophenyl	$C_{25}H_{18}N_4O_4Cl_2$	165	50	10.99	10.32
6	4-chlorophenyl	$C_{25}H_{18}N_4O_4Cl_2$	169	50	10.99	10.22
7	3,4-dibromo-2-hyroxy phenyl	$C_{25}H_{17}N_4O_5ClBr_2$	145	30	8.683	8.53

8	3,4-dichlorophenyl	$C_{25}H_{17}N_4O_4Cl_3$	135	40	10.30	10.31
9	3,4-dimethoxyphenyl	$C_{25}H_{23}N_4O_6Cl$	140	45	10.47	10.43
10	3,4-dimethoxy-5-nitrophenyl	$C_{27}H_{22}N_5O_8Cl$	148	35	12.29	12.01
11	4-Methoxyphenyl	$C_{26}H_{21}N_4O_5Cl$	130	40	11.10	10.98

[B] IR SPECTRAL STUDY (SHIMADZU-2245)

TYPE	Vibration Mode	Freq in cm-1		Reference
		Obs.	Reported	
Alkane $-CH_3$	-CHstr.(asym)	2965	2975-2950	28-29
	-C-Hstr.(sym)	2860	2880-2860	
$-CH_2$	-CHstr.(asym)	2795	2850-2765	
	-C-H sci.	1490	1480-1440	
	-C-H twisting	1255	1250	
Aromatic (1-4-disubst.)	-C-H str.	3055	3050-3030	
	-C=C- str.	1600	1615-1600	
		1525	1520-1480	
		1400	1417-1401	
	-C-H (oop) def.	830	832-802	
Amide -CO-NH-N-	NH.str.(asym.)	3350	3550-3250	
	-C-N. str. + -N-H def II band	1655	1655 - 1580	
	-C=O str.2º	1650	1680-1630	
	C-N vib.	1220	1220 - 1020	
	-C-Cl	650	830 – 540	
	-C=N str. Formazan	1620	1690-1580	
	-N=N- formazan	1575	1630-1575	
Coumarin moiety	-C=O α-lactonic ring	1725	1725-1730	
		1260	1220-1260	
β-Lactam ring	C=O (str.)	1715	1760-1660	
	C-Cl (str.)	750	830-540	

[C] MASS SPECTRA



ANTIMICROBIALACTIVITY4-METHYL-7-[α-(p-CHLOROPHENYLAZO)p METHOXYBENZALHYDRAZINO CARBONYLMETHOXY]COUMARIN.

Method :	**Cup-plate method(29, 30)**
Gram positive bacteria:	Bacillus Mageterium (2087)
	Staphylococcus citrus
Gram negative bacteria:	Escherecia Coli
	Salmonella Typhosa
Fungus	Aspergillus niger
Concentration	50µ gm
Solvent used	DimethylFormamide
Standard Drugs	Ampicilin; Chloramphenicol
	Norfloxacin; Griseofulvin

The nutrient agar broth & sterilized sabouraud's agar prepared by the usual method, was inocculated aseptically with 0.5ml of 24 hour old subculture of various bacteria in separate conical flasks at 40 -50 ºC and mixed well by gentle shaking . About 25 ml of agar broth was poured and evenly spread over sterilized Petri dish (13 cm in diameter) and allowed to set for 2 hours. The cups (10mm in diameter) were formed by help of the cork borer in agar medium and inoculated with various bacteria and fungi separately the cups were filled with 0.05ml (1mg/ml) of all

the test samples of azetidinones in DMF solution the plates were incubated at 37ºC for 24 hours and the control was also maintained with 0.05 ml of DMF in same way the Zones of inhibition were measured in mm. and recorded in table no-3

TABLE No. – 3

ANTI MICROBIAL ACTIVITY OF 4-METHYL-7-[α-(p-CHLOROPHENYLAZO)p-METHOXY BENZALHYDRAZINO CARBONYLMETHOXY]COUMARIN.

Sr. No.	COMPOUND	Zone of inhibition in mm.				
		Bacteria				Fungi
		B.maget	S.Citrus	E.coli	S.Typhosa	A.Niger
1	Phenyl	15	14	15	14	19
2	3-Aminophenyl	14	12	18	18	13
3	4-Aminophenyl	14	15	20	15	15
4	5-bromo-4-hydroxy-3 methoxyphenyl	12	21	20	17	19
5	2-chlorophenyl	15	18	15	15	14
6	4-chlorophenyl	14	13	17	17	15
7	3,4-dibromo-2-hyroxy phenyl	18	17	18	15	18
8	3,4-dichlorophenyl	14	16	16	15	14
9	3,4-dimethoxyphenyl	14	16	18	15	14
10	3,4-dimethoxy-5-nitrophenyl	13	16	19	15	15
11	4-Methoxyphenyl	14	16	17	14	15
12	Ampicillin	23	26	24	25	-
13	Chloramphenicol	27	22	21	23	-
14	Norfloxacin	22	27	25	27	-
15	Griseofulvin	-	-	-	-	24

[4] Result and Discussion : s

The compounds were screened for both gram positive and gram negative bacterias and Fungus by the Cup-plate method (29,30).
The compound no-2,4 and 6 were moderately active against S.Typhosa as compared with the standard drugs, for E.COLI compound no. 10 are moderately active and compound no- 3 and 4
shows similar activity as compared to standard drug. For gram positive bacteria S.Citrus bacteria compound 4showed good activity. And for B.Mageterium compound 7 showed moderate activity as compared to standard drug.

In case of antifungal activity against A.Niger almost all the compounds showed low activity other than compound no-1,4 and 18 showed moderate activity report comparing with the standard drug.

Acknowledgement

The authors are grateful to Department of Chemistry Saurashtra University Rajkot for providing total research facility and CDRI Lucknow for the various spectral analysis. Special thanks to the Trustees And Principals Of Institutes Shri M.D.SCIENCE COLLEGE PORBANDAR and Shri M.M.SCIENCE COLLEGE MORBI

REFERENCES :

1. Kamiyat., (Res. Lab. Fujisawa Pharm. Co.,Ltd. Osaka Japan), Kagaku N.,Pyoiki Zokan,112, 29-43, (1976) Chem. abstract 86, 16566a (1977).
2. Maffii G., (Cleptit S.P.A., Milan Italy), Chem. Abst. 53 ,20553 b (1959).
3. Maffii G. II Farmaco, 14 176-93 (1959).
4. Hotz. Roger D., Krenzer john U.S. Patent 3, 958, 974, Chem. Abst. 12, 697 (1917).
5. Bose A.K., Mannar M.S., Kapoor J.C., Sharma S.D., (Dept. of Chem. Eng.Stevens Inst. Technol.); Hoboken N. J., J.Med. Chem 17(5), 541-4(1974).
6. Bose P.k., J.Ind.Chem.Soc, 35: 367, (1958).
7. Sethna S.M.,Shah N.M., "Chem. Rev."36: 1(1945)
8. Wawzonek S., Heterocyclic Compounds Ed., Elder Field R.C., John Willey and Sons Inc. NewYork 2: 173, (1953).
9. Mohan R.R., Agarwal C., Misra, V.S. "Indian Drugs" 21g, 389-92(1984); Chem. Abst.101. 210928 P, (1984).

10. Chiarino Dario, Gramcini G.C., Viviana F. , Carenz Angelo, Chem. Abst. 110(13): 114824 P,(1989).
11. GuoHong Linghan D, Li Ming Hua, Zhongguo Yaoke Daxuew Xuebao 18(3) : 165-8 (1987); Chem. Abstr. 109; 54524 m (1988).
12. Elderfield R.C., Roy J., J. Med. Chem., 10: 918 (1957).
13. Castro S.W., Desouza A., Bemardino N., Pedido B., Chem. Abstr. 110: 135088 V (1989)
14. Ahluwalia Y.K. , Anand Saroj , Goyal m. , Neghipur G. A. , Bokin Baboi , 11(11) 622-26(1983) ; Chem. Abstr. 100: 191697 y (1984).
15. Gandolfi C.A., Tofanetti O. , Spinelli Silvano, Chem. Abstr. 105(15) : 133754 f (1986).
16. Chou T.Q.., Fu F. Y. , KooY.S. , J.Am.Chem. Soc. :70: 1765 (1948).
17. Rodhighiero P. , Manzini P. , Pastorini G., Guiotto A. , J. Hetro. Chem. 18(3) : 447 (1987).
18. Panigrahi A.K., Jesthi P.K., Rout M.K.; J. Proc. Inst. Chem. 38(4) 171-4 (1966); Chem.Abstr. 66 75876 (1967).
19. Weder F.W., Mercks Jahresbertichte 50 :88 (1936).
20. Shridhar D.R., Sarma C.R. Ram Bhagat Ind. J. Chem 22 B (8): 835-6,(1983); Chem. Abstr.100: 68126 (1983).
21. Umezawa H.,Kataoka M. ,Hosoi K.,Kawabe N., Ohno M.,Takeuchi T.; Chem.Abstr.83:206102 (1975).
22. Chen Jing Ting,Gao Chamglau, Jinxia Xu, Faming Zhuauli, Shenquing Gonkai,Chem.Abstr. 116 17209 m(1992)
23. Tiwari S.S., Kumar S., Ind. J. Applied Chem. 34 (3-4): 182-4 (1972).
24. Moffet R.B,Ibid,7:4466 (1954) U.S. 3156 697, (1954).
25. V.B.Gaur, V.H.Shah, A.R.Parikh; Ind.J.Hetr. Chem.: 1,141(1991).
26. V.S.Bhagwat,J.A.Parvate and M.N.Joshi,J.Ind.Chem. Soc.69;231(1992).
27. Yale H.C.,Lose V.K., Martin J.,J. Am. Chem. Soc. 75:1933(1953).
28. Poul V. M."Absorption Spectroscopy of organic Molecules" Addison –Wesley Pub. Co.,London 243-258 (1978).
29. Barry A.L., The Antimicrobial Susceptibility Test: Principle and Practices, Illus Lea and Febiger; Philadelphia. Pa. U.S.A.P-180-93 (1976); Chem.Abstr.64: 25183 (1977).
30. Brown A.G.,Roberts S. M.,Recent Advances in the chemistry of β-Lactam antibiotics. The Royal Society of Chemistry Buulington House London (U.K.) (1984).
31. Nesynov E.P., Pelkis P.S., Shvniger M.O., Mandrik T.P., Inst. Org . Khim. Kiev.USSR, Chem. Abst. 73 74153f,1970.
32. Plant .D., Zawistoweska, Eckstem Z.,Bul. Acad. Polen Sci, Ser, Sci. Chim 14(1) ; (1966); Chem Abstract. 61 36232, 363244h(1964).
33. Srivastava N;Bahadur S; Saxena M; Verma K.M;Chowdhry B; Ind. Jour. Chem.. Soc. 228(50)1068-69 (1983); Chem. Abst.100 1747285 (1984).

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