J. Biol. Chem. Chron. **2019, 5***(3),* 91-94
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Synthesis of Hydrazone Derivatives of Coumarin and Their Microbial Activities

A. P. Manake[1*], S. R. Patil[2] and R. S. Balaskar[3]

[1,3] Organic research laboratory, Department of Chemistry, Pratap College, Amalner (MS) INDIA
[2]M.G.S.M's A. S. C. College, Chopda. Tal-Chopda. Dist-Jalgaon, Maharashtra. INDIA

[*] Correspondence: E-mail: apmanake@gmail.com

(Received 10 Dec, 2018; Accepted 11 Jan, 2019; Published 18 Jan, 2019)

ABSTRACT: The hydrazone derivatives have been synthesized from available precursors like 2,4-dinitrophenyl hydrazine, and thiosemicarbazide in excellent yields in ethanol as a solvent without any catalyst. Formyl derivatives of coumarin have been synthesized by using HMTA. All these compounds have been screened for their preliminary potent microbial activities against both Gram-positive and Gram-negative bacteria.

Keywords: 7-hydroxy-4-methyl coumarin; HMTA; 2,4-DNP; thiosemicarbazide.

INTRODUCTION: Coumarin is a natural product found in some plant sources. Many plant kingdom contains some coumarin derivatives [1]. Many compounds of this moity has an aromatic odor and sweet fragrance. Hence it can used as a flavoring agent [2]. The coumarins are of great interest due to their biological properties [3]. Interesting compounds have been used for further backbone derivatization and use as novel precursor for therapeutic agents [4]. Coumarin and its derivatives have been found to have antimicrobial [5], antiviral [6], anti-inflammatory [7], antioxidant [8], anti-cancer [9], anticoagulant and enzyme inhibition activities [10].

Hence by knowing large applications of these heterocyclic compounds, it was planned to synthesize new coumarin derivatives at C8 by using Duff reaction and further hydrazone derivatives. Some compounds were screened for their antibacterial activity [11,12].

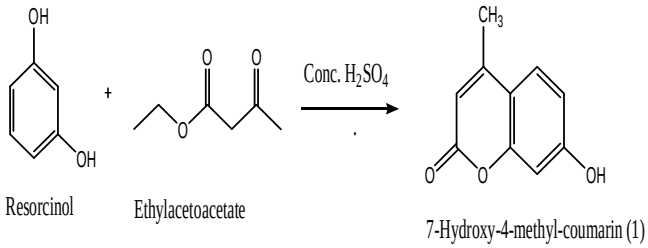
In medicinal chemistry these heterocyclic ring compounds have enormous significances. In view of this, we hope to prepare potent biologically active compound.

MATERIALS AND METHODS:

STAGE 1: *Synthesis of 7-hydroxy-4-methyl coumarin (1):*

Scheme-1: A solution of (0.01 mole) resorcinol and (0.01 mole) ethyl acetoacetate was added drop wise over a period of 30 min, with continuous stirring to (10 ml) of concentrated sulfuric acid in an ice bath so that the temperature of the mixture did not rise above 10˚C. The reaction mixture was reflux for half an hour and kept at room temperature for 3 hrs, and then poured with vigorous stirring into a mixture of ice and water. The precipitate was filtered off and washed with water, then after drying, recrystallized using ethanol. to get the pure product. Record the yield of product, Melting point of product & T.L.C. with their R.F. value.

Reaction:



Scheme-2: A solution of (0.01 mole) resorcinol and (0.01 mole) ethyl acetoacetate was added drop wise over a period of 30 min, with continuous stirring to (10 ml) of concentrated sulfuric acid in an ice bath so that the temperature of the mixture did not rise above 10˚C. The reaction mixture was kept overnight at room temperature without reflux, and then poured with vigorous stirring into a mixture of ice and water. The precipitate was filtered off and washed with water, then after drying, recrystallized using ethanol.

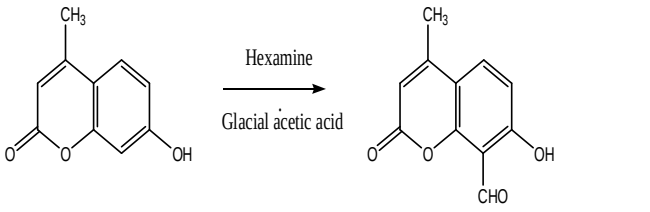
Table 1: Table showing Reaction condition, Time, Temperature and Yield of Compounds 1.

Compound	Time with temperature	Yield %	Reaction Conditions
1a	Reflux half hour & kept 3 Hrs at RT	79%	Conc. H_2SO_4, addition at 0^0C, Reflux, 3 hrs at RT.
1b	Overnight at RT	87%	Conc. H_2SO_4, addition at 0^0C, Overnight at RT. (No reflux Needed)

Table 2: Rf values, Yields and melting points of the synthesized compounds 1.

Compound	Observed m.p.(^{0}C)	Reported m.p. (^{0}C)	Yield %	Rf Value
1a	182	181-183	70	0.76
1b	179	181-183	87	0.84

STAGE II: ***Synthesis of 8-formyl-7-hydroxy-4-methyl coumarin (2):***



7-Hydroxy-4-methyl-coumarin

8-formyl-7-hydroxy-4-methylcoumarin (2)

Experimental Procedure: Mix 7-hydroxy-4-methyl-coumarin (0.025 moles) and Hexamethylenetetramine (0.07 moles) in glacial acetic acid (40 ml). Then mixture is heated at 85-90 C˚ on a water bath for 7 hrs. The hexamine adduct is formed. Then hydrolyzed it with 20% HCl (75 ml). The mixture heated again for 30 min. Cool the reaction mixture and extract with diethyl ether (50 ml) twice. The ether layer is evaporated by using a rotary evaporator. The yellow colored crystals obtained which was recrystallized from ethanol.

Table 3: Yield and Melting point.

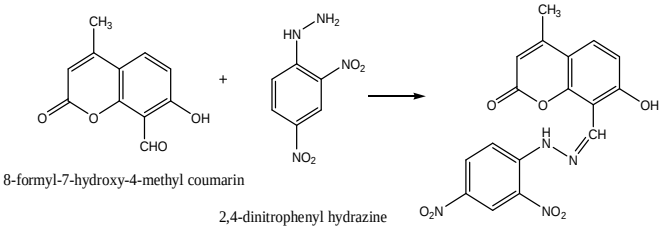
Sr. No.	Parameters	Observation
1	% yield	22%
2	Melting point	177-179 ˚C
3	Solubility	Ethanol
4	Reaction time	7 hours

STAGE III: ***Synthesis of hydrazone derivatives 3a-b:***

Experimental procedure: Reflux Compound (2), (0.01 mole) and hydrazine derivatives (0.01 mole); (2,4-dinitrophenyl hydrazine, and thiosemicarbazide) in round bottom flask for 1 hour in the presence of ethanol. The precipitate is then filtered, dried and recrystallized from ethanol.

Synthesis of 8-((2-(2,4-dinitrophenyl) hydrazono) methyl)-7-hydroxy-4-methyl coumarin (3a):

Reaction:



8-formyl-7-hydroxy-4-methyl coumarin

2,4-dinitrophenyl hydrazine

hydrazone derivative of 2,4 DNP (3a)

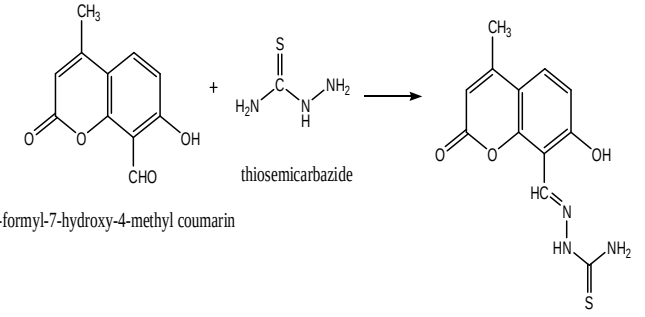
Intense orange colored precipitate.

Table 4: Yield and Melting point.

Sr. No.	parameters	Observation
1	% yield	85%
2	Melting point	287-290 ˚C
3	Solubility	Ethanol
4	Reaction time	1 hour

Synthesis of 2-((7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl) methylene) hydrazinecarbothioamide (3b):

Reaction:



8-formyl-7-hydroxy-4-methyl coumarin

thiosemicarbazide

hydrazone derivative of thiosemicarbazide (3b)

Faint yellow colored precipitate

Table 5: Yield and Melting point.

Sr. No.	parameters	Observation
1	% yield	90%
2	Melting point	280-284˚C
3	Solubility	Ethanol
4	Reaction time	1 hour

Microbial Activity Against Given Compounds: Microbial activity of synthesized chemical compounds was studied by “microbial assay” process. The

chromenes (5c-e) obtained were preliminarly evaluated for their in vitro antibacterial activity against a narrow spectrum of bacterial species procured from the Laboratory of Microbiology (Microbiology. Dept., Faculty of Science, Pratap College, Amalner).

The formyl derivative and compounds were selected for microbial activity namely the product of condensation with 2,4: DNP.

Table 6: Microbial Activities.

Name of the compound	Culture	Diameter	Result
Coumarin	*E-coli*	10mm	positive
	Bacillus subtilis	11mm	positive
	Staphillococcus aureus	13mm	positive
	Protius	10mm	positive
Formyl derivative	*Bacillus subtilis*	11mm	positive
2,4:DNP derivative (3a)	*E-coli*	--	positive
	Bacillus Subtilis	13mm	positive
	Staphillococcus aureus	10mm	positive
	Protius	10mm	positive

Table 7: IR spectral analysis.

Compd	IR (cm^{-1})
1	3157(υ OH), 1790 (υ C=O), 1600 (υ C=C).
2	3435 (υ OH), 1744 (υ C=O, lactone), 1645 (υ C=O, aldehyde), 1595 (υ C=C).
3a	3422 (υ OH), 3232 (υ N-H), 1740 (υ C=O, lactone), 1610 (υ C=N), 1568 (υ C=C aromatic)
3b	3398 (υ OH), 3390 and 3280 (υ NH2), 3184 (υ N-H), 1730 (υ C=O, lactone), 1598 (υ C=N), 1458 (υ C=C)Aromatic

^{1}H NMR Spectral Analysis:

Compound 1: 1H NMR (300MHz, DMSO): δ 2.49 (s, 3H, C4-CH3), 6.31 (s,1H, C3-H), 6.92 (d, 1H, C6-H, J=9.0 Hz), 6.94 (s, 1H, C8-H), 7.57 (d, 1H, C5-H, J=9.0 Hz).

Compound 2: 1H NMR (300 MHz, DMSO): δ 2.44 (s, 3H, C4-CH3), 6.22 (s, 1H, C3-H), 6.90-6.93 (d, 1H, C6-H, J=9 Hz), 7.73-7.76 (d, 1H, C5-H, J=9 Hz), 10.63 (s, 1H, HCO), 12.28 (s, 1H, OH).

Compound 3a: 1H NMR (300 MHz, DMSO): δ 1.78 (s, 3H, C4-CH3), 6.01 (s, 1H, C3-H), 6.50-6.63 (d, 1H, C6-H, J=9 Hz), 7.13-7.26 (d, 1H, C5-H, J=9 Hz), 08.13 (s, 1H, H of H-C=N), 6.99-7.01 (d, 1H), 8.30-8.41 (d, 1H), 8.88-8.92 (s, 1H).

Compound 3b: 1H NMR (300 MHz, DMSO): δ 1.72 (s, 3H, C4-CH3), 1.99-2.01 (s, 2H, NH_2), 6.01 (s, 1H, C3-H), 6.50-6.63 (d, 1H, C6-H, J=9 Hz), 7.13-7.26 (d, 1H, C5-H, J=9 Hz), 08.13 (s, 1H, H of H-C=N).

RESULTS AND DISCUSSION: Here we synthesize the coumarin derivatives, (Scheme-1 and Scheme-2), 7-hydroxy-4-methyl coumarin (1) without refluxing and keeping overnight just at room temperature (scheme-2). Here we insert the formyl group (CHO) at the carbon 8 and confirmed by IR band of C=O stretching of aldehyde at 1645 cm^{-1} and ^{1}H NMR displays 10.63 δ (s, 1H) confirms the CHO function at carbon number 8 of 7-hydroxy-4-methyl coumarin. We prepare the Hydrazone derivatives of coumarin (3a-b) by refluxing compound (2) with different hydrazines in ethanol as a solvent. IR band of C=N stretching obtained in the range of (1598-1610 cm^{-1}) and ^{1}H NMR displays 08.13 δ (s, 1H, H of H-C=N) which confirms hydrazone derivative. Our all synthesized compounds show considerable antibacterial activity *in vitro*. Among all the derivatives, compound (3a) and formyl derivative shows the highest rate of inhibition against *Bacillus Subtilis*, while coumarin shows the greatest anti-bacterial activity against *Staphillococcus aureus.*

CONCLUSION: We synthesized the different derivatives of coumarin by condensation of carbonyl group with NH_2 of Hydrazine and carbazide. All the synthesized compounds display substantial microbial activities against both Gram-positive and Gram-negative bacteria.

REFERENCES:

1. Sentha S. M. (1945) Synthesis of coumarin and 4-methyl coumarin. *Chem Rev*, 36:2.
2. Darren Holling, Graham Sandford, Andrei S Batsanov, Dmitril S Yufit, Judith AK Howard. (2005) Direct fluorination of coumarin, 6-methylcoumarin, and 7-alkoxy-coumarins, *J Fluorine Chem*, 126(9-10), 1377-83.
3. Pangal A, Gazge M, Mane V, Shaikh J. A. (2013) Various pharmacological aspects of coumarin derivatives: a review, *Int J Pharm Biol*, 2(6), 168-94.
4. Lin P. Y., Yeh K. S., Su C. L., Sheu S. Y., Chen T., Ou K. L., *et al.* (2012) Synthesis and antibacterial activities of novel 4-hydroxy-7-hydroxy-and 3- carboxycoumarin derivatives, *Molecules*, 17, 10846-63.
5. Swamy B. H. M. J., Nandini H. G. M., Pramod N. (2013) Synthesis, characterization and antifungal activity of coumarin carbohydrazide containing

quinoline derivatives, *Int J Res Pharm Nan Sci*, 2(2), 195-2.

6. Darla M. M., Krishna B. S., Rao K. U., Reddy N. B., Srivash M. K., Adeppa K, *et al.* (2015) Synthesis and bio-evaluation of novel 7-hydroxy coumarin derivatives via Knoevenagel reaction, *Res Chem Intermed*, 41, 1115-33.

7. Al-Kawkabani A., Boutemeur-Kheddis B., Makhloufi-Chebli M., Hamdi M., Talhi O., Silva A. M. S. (2013) Synthesis of novel 2H,8H-pyrano [2,3-f] chromene-2,8-diones from 8-formyl-7-hydroxy-4- methylcoumarin, *Tetrahedron Lett*, 54, 5111–4.

8. Aly M. R. E. S., Fodah H. A. E. R., Saleh S. Y. (2014) Antiobesity, antioxidant and cytotoxicity activities of newly synthesized chalcone derivatives and their metal complexes, *Eur J Med Chem*, 76, 517-30.

9. Syam S., Abdelwahab S. I., Al-Mamary M. A., Mohan S. (2012) Synthesis of chalcones with anticancer activities, *Molecules*, 17, 6179-95.

10. Singh I., Kaur H., Kumar S., Kumar A., Lata S., Kumar A. (2010) Synthesis of new coumarin derivatives as antibacterial agents, *Int J Chem Tech Res*, 2, 1745-52.

11. Mantri N., Jadon G. (2013) Two Synthesized mannich bases of 7-hydroxy-4-methyl coumarin with a different secondary amine, *Int J Adv Res Pharm Biosci*, 3(2), 117-26.

12. Manidhar D. M., Rao K. U. M., Reddy N. B., Sundar C. S., Reddy C. S. (2012) Synthesis of new 8-formyl-4 methyl-7-hydroxy coumarin derivatives, *J Korean Chem Soc*, 56(4), 459-63.