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MANNICH BASES OF 7-HYDROXY-4 -METHYL AND 4, 6, 7-TRIMETHYL COUMARINS AS POTENTIAL FUNGICIDES FOR *ASPERGILLUS FLAVUS* AND *PYRRICULARIA ORZAE*

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Keywords:

Coumarin, Mannich bases, Amino acids, Aldehydes, Fungicidal activities, *Aspergillus flavus* and *Pyricularia oryzae*

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ABSTRACT: Coumarin and its derivatives are widely used as scaffolds in synthesizing new heterocyclic systems. Numerous approaches especially involving nano-particle catalysts, have been developed to get new bioactive coumarin derivatives endowed with pharmacological and biological activities. The present work describes the reactivity and the new strategies for the synthesis of coumarin and its derivatives reported in the literature and their biological properties. Coumarins possess a number of biological activities like Anticoagulant, Antimicrobial, Anti-inflammatory, Analgesic, Antioxidant, Anticancer, Antiviral, Anti-malarial *etc*. Coumarin belongs to a group as benzopyrones, which consists of a benzene ring joined to a pyrone nucleus [12]. Coumarins (2H-1-benzopyran-2-ones) are important oxygen-containing fused heterocycles used in drugs and dyes. A number of coumarin derivatives have been reported to have diverse biological activities like fungicidal[1-4], molluscicidal [2], antihelminthic [5, 6] and CNS [7] stimulants. Mannich Bases of some heterocyclic compounds are also reported Bioactive [8, 9]. In view of this Mannich bases of 7 – hydroxyl – 4 – methyl coumrin and 4, 6, 7–trimethyl coumaryl incorporating various DL–amino acids as a basic component were synthesized and evaluated for their fungitoxicity against *A. flavus* and *P. oryzae*.

INTRODUCTION: Coumarins (2H-1-benzopyran -2-ones) are important oxygen-containing fused heterocycles used in drugs [13] and dyes. Coumarins be bound their class name to ‘coumarou’ the vernacular name of the Tonka bean (Dipteryx odorata wild, Fabaceae), from which coumarin was isolated in 1820. They are the family of lactones containing benzopyrone skeletal framework that have enjoyed isolation from the plant and total synthesis in the laboratory. The present work describes the reactivity and the new strategies for synthesizing coumarin and its derivatives reported in the literature and their biological properties [14].

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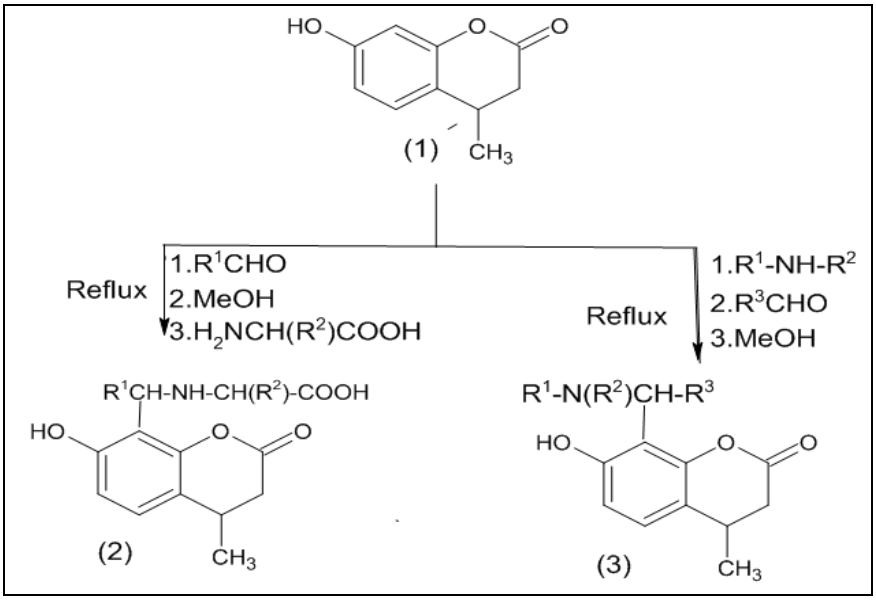
In view of this Mannich bases of 7– hydroxyl – 4 – methyl coumrin and 4,6,7–trimethyl coumaryl incorporating various DL–amino acids as a basic component was synthesized and evaluated for their fungitoxicity against *A. flavus* and *P. oryzae*

MATERIAL AND METHODS: The required 7– hydroxyl – 4 – methyl coumarin (1) was prepared following the method of Pachmann and diusherg [10] by the condensation of ethyl acetoacetate with resorcinol in the presence of Conc. H_2SO_4.

Synthesis of 7-Hydroxy-4-Methyl Coumarin: About 150 ml. of conc. H_2SO_4 in a 500 ml beaker was stirred with external ice water cooling until the temperature of acid became about 5°C – 10°C, 37 gm of powdered resorcinol was added to 45 ml. of ethyl acetoacetate until a complete solution was obtained. Then this solution was added slowly to H_2SO_4. In such a way, the temperature did not rise above 100C, and the stirring was continued for ½ an hour. The mixture is poured into the ice/cold

water & the solid product is separated, filtered out and dried. Then the crude product was recrystallized from absolute ethanol. The resultant Yields: 85%, Melting point: 192°C, Molecular Formula: $C_{10}H_8O_3$, Molecular Mass: 176.17 and Solubility: Methanol, Ethanol, and Pyridine. Condensation of (1**)** with DL – amino acids or aldehydes in the presence of formaldehyde and methanol, furnished Mannich bases (2) and (3), respectively as shown in **Scheme I.**



SCHEME I.

The structural assignments of the synthesized compounds have been established by elemental analysis and spectral data. IR spectra recorded on a Perkin-Elmer-15 spectrophotometer and 90 MHz PMR (DMSO d_6) on Varian EM 390 spectrometer (Chemical shift in δ-ppm). Melting points were taken open glass capillary and are uncorrected.

Synthesis of test Compounds: N-[(7-hydroxy – 4 – methyl coumarin – 8 – yl)] methyl glycine (2A): A mixture of N-(7-hydroxy–4–methyl coumarin (0.01M), glycine (0.01M) and formaldehyde (0.01M) in methanol (20 Ml) was reflux for 5 hours. Poured the mixture into ice cold water. Solid thus obtained was filtered, washed with water, and recrystallized from ethanol.

Yield 90%, m.p. 203°C, IR (in KBr) $cm^{-1}\nu_{max}$ 3470 (OH), 3200 (NH), 1670 (C=O), 1570, 1555, 1430, 1390 (aromatic ring); PMR (DMSO-d_6): δ (s, 3H, CH_3), 3.9 (s, 2H, CH_2NH), 6.6 – 7.8 (m, 4H, ArH and NH). Other compounds 2b – 2j were also synthesized similarly in **Table 1.**

TABLE 1: PHYSICAL CHARACTERISATION DATA OF COMPOUND (2)

Compound	R^1	R^2	R^3	Yield (%)	m.p. (^{0}C)
2a	H	H	--	90	203
2b	CH_3	H	--	69	210
2c	CH_3	CH_3	--	65	161
2d	C_6H_5	CH_3	--	70	167
2e	CH_3	$CH_2CH(CH_3)_2$	--	80	180
2f	C_6H_5	$-CH_2-C_6H_4-OH$	--	62	168
2g	H	CH_3	--	85	147
2h	C_6H_5	H	--	61	164
2i	H	$-CH_2-C_6H_4-OH$	--	55	211
2j	CH_3	$-CH_2-C_6H_4-OH$	OH	69	62

N-[(7 – hydroxyl – 4 – methyl coumarin – 8 – yl)] methyl/ethyl substituted amines (3A) : N-(7-hydroxy – 4 – methyl coumarin (0.01M), dimethyl amine (0.01M) and formaldehyde (0.01M) in methanol (25 Ml) and water (2 Ml) were refluxed for 5 hours, the resulting mixture was cooled and poured into water and recrystallized from ethanol. Yield 72%, m.p. 163°C, IR (in KBr): 3480 (OH),

1670 (C=O), 1600, 1580, 1440 (aromatic ring); PMR (DMSO- d_6) δ 1.7 (s, 3H, CH_3), 2.6 (s, 6H, CH_3-N-CH_3), 3.1 (s, 2H, CH_2-N), 6.1 – 6.9 (m, 3H, ArH). Other compounds 3b – 3h were synthesized by similar methods **Table 2.**

TABLE 2: PHYSICAL CHARACTERISATION DATA OF COMPOUND (3)

Compound	R^1	R^2	R^3	Yield (%)	m.p. (^{0}C)
3a	H	CH_3	CH_3	69	166
3b	CH_3	CH_3	CH_3	62	161
3c	H	C_2H_5	C_2H_5	63	173
3d	CH_3	C_2H_5	C_2H_5	70	165
3e	H	C_6H_5	C_6H_5	62	197
3f	CH_3	C_6H_5	C_6H_5	64	162
3g	H	CH_3	C_6H_5	60	178
3h	CH_3	CH_3	C_6H_5	61	123

Fungitoxicity: The fungicidal activity was evaluated against *Aspergillus flavus* and *Pyricularia oryzae* by Agar growth technique [11] at 100 and 10 ppm concentration by adding a solution of the test compounds in acetone: water (20: 80; v/v) mixture to pre-sterilized Petri-dishes containing Czepek's agar and mixing thoroughly. One week old culture of the test fungi was inoculated in centre of each Petri-dish. The bio-assay was done in three replicates. The plates were incubated at 28±2°C for 7 days. The percentage inhibition of the mycelia growth or spore germination was calculated by the following formula.

$$\% \text{ Inhibition} = C - T / C \times 100$$

Where, C = average diameter (in mm) of the fungal colony in control plates, T = average diameter (in mm) of the fungal colony in treated plates,

ED_{50} – Values were calculated by the log probability method. The commercial fungicide Dithane M-45 was maintained as a reference for comparison in **Table 3.**

TABLE 3: FUNGICIDAL ACTIVITY OF COMPOUNDS (2 & 3)

Compound	R^1	R^2	R^3	ED_{50} (ppm) against	
				Aspergilus flavus	*Pyricularia oryzae*
2a	H	H	--	132	162
2b	CH_3	H	--	95	100
2c	CH_3	CH_3	--	92	94
2d	C_6H_5	CH_3	--	90	82
2e	CH_3	$CH_2CH(CH_3)_2$	--	18	80
2f	C_6H_5	-CH_2-C_6H_4-OH	--	10	28
2g	H	CH_3	--	142	205
2h	C_6H_5	H	--	95	130
2i	H	-CH_2-C_6H_4-OH	--	26	29
2j	CH_3	-CH_2-C_6H_4-OH	OH	164	23
3a	H	CH_3	CH_3	101	94
3b	CH_3	CH_3	CH_3	130	185
3c	H	C_2H_5	C_2H_5	95	96
3d	CH_3	C_2H_5	C_2H_5	91	82
3e	H	C_6H_5	C_6H_5	130	185
3f	CH_3	C_6H_5	C_6H_5	88	80
3g	H	CH_3	C_6H_5	91	82
3h	CH_3	CH_3	C_6H_5	142	180
Dithane M-45				10	13

RESULTS AND DISCUSSIONS:

Fungicidal Activity: The fungicidal activity of the test compounds of type 2 was comparatively more active than the compound of type 3. The most active compounds were 2e, 2f, 2i, 2j, 3d and 3g. By comparing the data of compound numbers 2 and 3, it was clear that amino acid derivatives played an important role. The presence of groups like –CH_3, and CH_2-CH-$(CH_3)_2$ accounted for their fungicidal activity.

The most active compounds 2e, 2f, 2i, 2j, 3d and 3g showed comparatively inferior fungi toxicity than the commercial fungicide Dithane M-45 at 100 and 10 ppm against both the test fungi. The activity decrease on dilution.

CONCLUSIONS: From the above results and discussion, Mannich bases with coumarin and its derivatives enhance the fungicidal activities. The presence of groups like $–CH_3$ and CH_2-CH-$(CH_3)_2$ accounted for their fungicidal activity.

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REFERENCES:

1. Sengupta AK, Sen S and Srivastava V: Synthesis of coumarin derivatives as possible antifungal and antibacterial agents. JICh Society 1989; 66(80): 710-716.
2. Giri S, Mishra AK: Fungicidal and Molluscicidal activity of some 3-substituted-4-hydroxy coumarin derivatives. J. Agriculture and Food Chemistry 1984; 32(4): 759-762.
3. Giri S, Sharan P and Nizamuddin: Synthesis of some coumarinyl-2-azilidinones as potential anti fungal agents. Agric Biol Chem 1989; 53(4): 1153.
4. Khan M H, Alauddin S and Nizamuddin: Fungi toxicity of Mannich bases of 7-hydroxy-4-methyl coumarin. Prsticide Research J 1998; 10(1): 105-108.
5. Bayer AG and Verwendung Von: 3-carbomoyl-4-hydroxy coumarin. Eur Pat Appl 0241834A, 1987; 21978.
6. Upjohn Company. Anthelmintic and Anti cocidal 3-carbomoyl-4hydroxy coumarin, method of use and composition. PCT International 1992; Appl WO 92/06083.
7. Gupta VN, Sharma BR and Arora RB: Pharmacologically active coumarin derivatives Mannich bases from umbelliferone and 4 – methyl umbelliferone. J Sci Ind Research 1961; 20: 300.
8. Shorts JH and Ours CW: Use of amino acids in Mannich reaction. J Heterocycl Chem 1975; 12: 869-876.
9. Khan MH and Giri S: Synthesis of 8-[(5’-aryl-1’,3’,4’-thiadiazol-2’-yl)aminomethyl]-7-hydroxy/acetoxy-4methyl coumarins as possible fungicides. IJC 1993; 32: 984-85.
10. Singh PR, Gupta DS and Bajpai KS: Experiments Organic Chemistry, Tata Mc Graw Hill Publishing Company Ltd. New Delhi 1980; 1: 182.
11. Horsfall JG: Quantitative Bioassay of fungicides in laboratory. Bot Rew 1945; 11: 357.
12. Sahoo SS, Shukla S, Nandy S and Sahoo HB: Synthesis of Novel coumarin derivatives and its biological evaluations. Uro J Exp Bio 2012; 2(4): 899-908.
13. Dubovik IP and Garazd M: Modified Coumarins:Synthesis of Aloperine containing Mannich bases of 7 – Hydroxycoumarins. CNC 2017; 53(3): 444-47.
14. Gul HI, Demistas A, Ucar G, Taslimi P and Gulcin I: Synthesis of Mannich bases by two different methods and Evaluation of their Acetylcholine Esterase and Carbonic Anhydrase inhibitory activities. Letters in Drug Design & Discovery 2017; 14: 5.

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