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SYNTHESIS OF SOME COUMARIN DERIVATIVES CONTAINING AZOMETHINE FUNTIONALITY AND EVALUATION OF THEIR ANTIOXIDANT POTENTIAL

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ABSTRACT:
In the current study, four new 3-acetyl-6-bromocoumarin hydrazones (**3a-3d**) were synthesized, and their structures were characterized using FTIR, ^{1}H-NMR, and HRMS techniques. The antioxidant activity of these hydrazones was evaluated using the DPPH radical scavenging method. Among them, hydrazones **3c** and **3d** exhibited significant antioxidant activity, with noteworthy IC_{50} values compared to the standard antioxidant, ascorbic acid. These results may be attributed to the presence of various functional groups, which could have enhanced the activity of **3c** and **3d**. Overall, the findings suggest that these compounds could serve as promising lead structures for the development of new drugs.

KEYWORDS: Coumarin, Bromocoumarin, Hydrazone, Antioxidant, DPPH.

INTRODUCTION:

In recent years, many researchers have focused on Schiff bases as a significant class of therapeutic molecules with a vast potential for biological efficacies like antibacterial, anticancer, anticonvulsant, and antioxidant properties[i-vi]. On the other hand, coumarins have attracted considerable attention due to their wide spectrum of pharmacological and biological activities such as anticoagulant and antiplatelet aggregation[vii], antitumor[vii], antifungal[ix], antiviral[x], antibacterial[xi-xii] and anti-inflammatory[xiii] agents. 3-Acetyl-6-bromocoumarin is a versatile compound known for its unique properties and applications in various fields, including pharmaceuticals and organic synthesis. Researchers have utilized 3-acetyl-6-bromocoumarin in the development of novel anti-cancer agents and anti-inflammatory drugs, showcasing its potential in medicinal chemistry[xiv]. Hydrazones or azomethines or imines possess -NHN = CH- and constitute an important class of compounds for new drug development[xv]. A number of researchers have synthesized and evaluated the biological activities of hydrazones. These compounds possess diverse biological and pharmacological properties[xvi] such as antimicrobial, anti-inflammatory, analgesic, antifungal, antitubercular,

antiviral, anticancer, antiplatelet, antimalarial, anticonvulsant, antihelmintic, antiprotozoal etc[xv-xvi]. Inspired by these observations, we became interested in synthesis of new Schiff bases **(3a-3d)** by using 3-acetyl-6-bromocoumarin. In this work we condensed 3-acetyl-6-bromocoumarin with different aromatic hydrazides under acidic condition. The synthesized Schiff bases were characterized by different analytical techniques like FTIR, HRMS and 1H-NMR. Further, these bases were evaluated for antioxidant potential by using DPPH radical scavenging method and the compounds **3c** and **3d** were found to show good antioxidant activity.

EXPERIMENTAL:

Syntheses of 3-acetyl-6-bromocoumarin Schiff bases:

Solvents for synthesis were reagent grade and dried by standard procedures. The starting materials such as 5-bromosalicyladehyde and different aromatic hydrazides were obtained from Sigma-Aldrich chemicals and piperidine. acetone, methanol, ethanol and Trifluoroacetic acid (TFA) were obtained from Loba Chemical Limited, India. Melting points of as synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus. The FTIR, ^{1}H-NMR and HRMS were obtained from Central Instrumentation Facility, Savitribai Phule Pune University (SPPU), Pune. The synthetic methodology employed for the synthesis of target compounds **(3a-3d)** is illustrated in **Figure 1**.

Piperidine (5 mol%) was added to a solution of 5-bromo salicylaldehyde derivative (5 mmol) and ethyl acetoacetate (5 mmol) in ethanol (20 mL) under stirring at room temperature for solid formed. After cooling, the precipitate was filtered and washed with cold ethanol to afford pure product **(1)** in good yield. To the solution of **(1**, 1 eq.**)** and different aromatic hydazides (1eq) in ethanol with 5-6 drops of trifluoroacetic acid were added and the mixture was stirred at room temperature till the completion of reaction monitored with TLC and then reaction mixture was poured into the cold water. The solid obtained was filtered and washed with cold ethanol and recrystallized from ethanol.

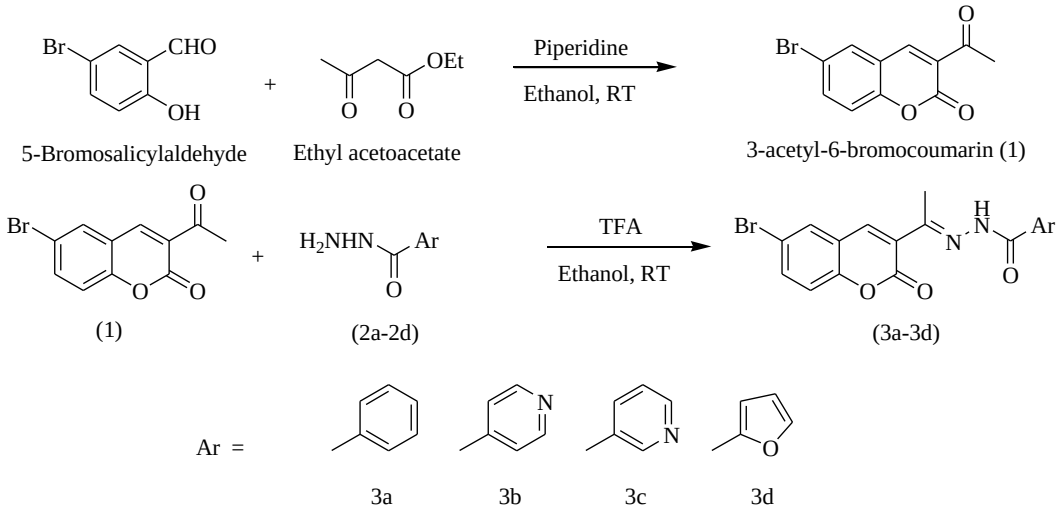

Figure 1: Synthesis of 3-acetyl-6-bromocoumarin Schiff bases

General Procedure for Antioxidant activity: - For the antioxidant assays, all compounds were dissolved in ethanol and a series of concentration-dependent dilutions were made. Standard chemicals were used for comparison. The free radical scavenging activity of the samples was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl). For this assay 3 mL of sample (in different concentrations) was added to 2 mL of a solution of DPPH in ethanol (0.004%). In order to subtract the absorbance promoted by the staining of the sample, a solution

of methanol (2.0 mL) and the sample (3.0 mL) was used as the blank (A_{blank}). A solution of DPPH (2.0 mL) and methanol (3.0 mL) was used as the control ($A_{control}$) which is regarded as 100% of DPPH. The absorbance of the resulting solution (A_{sample}) was measured after 30 min at 517 nm and converted into percentage of antioxidant activity using the Equation:

$$\% \text{ Radical scavenging activity } = \frac{(A_{control} - A_{sample})}{A_{control}} \text{ x } 100$$

RESULTS AND DISCUSSIONS

Chemistry:

Melting points of the synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus and are uncorrected. IR spectra were recorded by "FT- IR Jasco" spectrometer at our centre. The H^1-NMR and HRMS spectra were obtained from CIF, Savitribai Phule Pune University, Pune. The formation of **(1)** was confirmed from the physical constants as reported in the literature and the structures of the synthesized compounds were established on the basis of physical and spectral data. The detailed physical and spectral properties are summarized in **Table 1**.

Table 1: Characterization data for 3-acetyl-6-bromocoumarin Schiff bases

Sr. No.	Code	M. P. °C & Colour	% Yield	Spectral Properties
1	**3a**	320-322 Pale Yellow	85	**FTIR (cm^{-1}):** 3042.66 (-NH), 1703.97 (Lactone, >C=O), 1681.73 (>C=O), 1547.95(-C=N-), 1556.92-1485.36 (Aromatic, C-C), 1286.56 (-C-O-C-). **H^1-NMR** (*d-$CDCl_3$*) (δ, ppm,): 9.08 (s, -NH, 1H), 8.40 (s, olefinic, 1H), 7.46-7.886 (m, 8H), 2.72 (s, -CH_3, 3H). **HRMS** (EI^+) : 385.01 ($M+H^+$)
2	**3b**	232-234 Orange	84	**FTIR (cm^{-1}):** 3040.66 (-NH), 1783.06 (Lactone, >C=O), 1671.29 (>C=O), 1601.38 (-C=N-), 1542.62-1471.22 (Aromatic, C-C), 1225.30-1198.09 (-C-O-C-). **H^1-NMR** (*d-$CDCl_3$*) (δ, ppm,): 9.08 (s, -NH, 1H), 8.40 (s, olefinic, 1H), 7.46-7.886 (m, 8H), 2.72 (s, -CH_3, 3H). **HRMS** (EI^+) : 407.99 ($M+Na^+$)
3	**3c**	240-242 Yellow	78	**FTIR (cm^{-1}):** 3047.66 (-NH), 1782.82 (Lactone, >C=O), 1675.79 (>C=O), 1631.37 (-C=N-), 1583.59-1470.75 (Aromatic, C-C), 1230.57-1188.91 (-C-O-C-). **H^1-NMR** (*d-$CDCl_3$*) (δ, ppm,): δ 11.06 (s, -OH, 1H), 9.06 (s, -NH, 1H), 8.60 (s, olefinic, 1H), 8.29 (d, J=8Hz, 1H), 8.22 (d, J=2.5Hz, 1H), 7.89 (d, J=2.5Hz, 1H), 7.80 (d, J=8Hz, 1H), 7.61 (dd, J=4.5 and 3Hz, 1H), 7.45 (d, J=8Hz, 1H), 2.58 (s, -CH_3, 3H). **HRMS** (EI^+) : 386.01 ($M+H^+$)
4	**3d**	254-256 Pale Yellow	80.2	**FTIR (cm^{-1}):** 3040.70 (-NH), 1728.48 (Lactone, >C=O), 1671.13 (>C=O), 1598.83 (-C=N-), 1541.40-1470.21 (Aromatic, C-C), 1285.42 (-C-O-C-). **H^1-NMR** (*d-$CDCl_3$*) (δ, ppm,): δ 8.40 (s, -NH, 1H), 8.40 (s, olefinic, 1H), 7.78 (d, J=2.5 Hz, 1H), 7.74 (d, J = 2 Hz, 1H), 7.72 (d, J = 2 Hz, 2H), 7.280 (d, 1H), 7.26 (d, 1H), 2.72 (s, -CH_3, 3H). **HRMS** (EI^+) : 374.99 ($M+H^+$)

Results of Anti-oxidant Activity: -

Reactive oxygen and nitrogen species play a key role in generating free radicals, which can cause severe oxidative damage to biomolecules such as DNA, lipids, and proteins, potentially transforming healthy cells into cancerous ones[xvii]. Consequently, research has focused on developing novel heterocyclic compounds with antioxidant properties due to their pharmaceutical significance, aiming to design less toxic and more potent analogs of natural antioxidants. In this study, new coumarin hydrazones were synthesized by combining antioxidant-active coumarin with aromatic hydrazides. The antioxidant potential of the synthesized hydrazones (3a-3d) was assessed using the DPPH radical scavenging method. Briefly, 2 mL of the sample at varying concentrations was mixed with 3 mL of a 0.004% DPPH solution in ethanol. After 30 minutes, the absorbance of the solution was measured at 517 nm and converted into a percentage of antioxidant activity. The obtained results after drug treatment are summarized in **Table 2**.

Table 2: Results of % DPPH Radical Scavenging for new coumarin derivatives

Drug Concentration (µg/ml)	**ASC**	**3a**	**3b**	**3c**	**3d**
0	0	0	0	0	0
1.95	1.09	4.67	8.18	4.53	2.78
3.9	6.13	5.42	14.5	12.11	15.99
7.81	24.07	16.31	21.45	29.19	25.91
15.63	47.92	46.83	35.8	40.58	34.39
31.25	50.98	62.56	54.55	58.9	51.18
62.5	53.17	62.79	57.59	60.29	53.71
125	70.02	65.52	68.75	64.82	65.91
250	73.52	68.22	73.06	65.02	67.43
500	73.96	68.62	73.35	66.42	67.68
1000	75.49	68.69	79.48	68.32	69.79
IC50	**7.41**	**12.30**	**18.09**	**9.49**	**9.24**

The graph of % scavenging activity versus concentration of synthesized analogs was plotted and presented in **Figure 2**. The IC_{50} concentration values were determined from an online source (http://www.ic50.tk). The scavenging activity of the test compounds was compared to the standard antioxidant, ascorbic acid (ASC). An increase in compound concentration corresponded to an increase in radical scavenging activity. Among the tested hydrazones, compounds **3c** and **3d** demonstrated the most potent antioxidant activity, with IC50 values of 9.49 and 9.24 µg/ml, respectively. In comparison, hydrazones **3a** and **3b** exhibited scavenging activity with IC50 values of 12.30 and 18.09 µg/ml, respectively. The natural antioxidant ascorbic acid showed remarkable activity with an IC_{50} of 7.41 µg/ml. The antioxidant activity, which is attributed to a compound's ability to donate electrons or protons, depends on the presence of electron-donating groups (-OH and -NH) and substitutions on the aromatic ring. Consequently, the modified coumarin compounds 3c and 3d exhibited strong antioxidant activity, likely facilitated by their structure and extended π-electron conjugation.

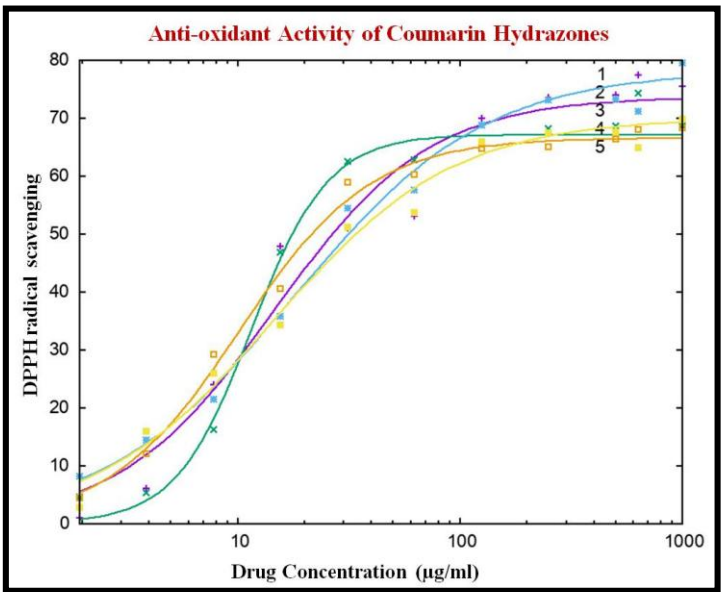

Figure 2: % DPPH radical scavenging activity curves of coumarin hydrazones (1 = Ascorbic acid, 2 = 3a, 3 = 3b, 4 = 3c, 5 = 3d)

CONCLUSION:

In this study, we successfully synthesized biologically active 3-acetyl-6-bromocoumarin hydrazones (**3a-3d**), and their structures were characterized and confirmed using FTIR, ^{1}H-NMR, and HRMS techniques. During the evaluation of antioxidant activity, hydrazones **3c** and **3d** exhibited notable antioxidant potential with appreciable IC50 values. In contrast, hydrazones **3a** and **3b** showed moderate activity compared to the standard antioxidant, ascorbic acid. These findings can be attributed to the presence of various functional groups, such as azomethine, -NH, nicotinyl, and furoyl groups, which likely enhance the activity of 3c and 3d. Overall, these results suggest that these compounds could serve as promising lead structures for the development of new drugs.

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