



**SYNTHESIS OF MONO AND DINITRO SCAFFOLDS OF 4-BROMOMETHYL
COUMARINS AND THEIR ANTIMICROBIAL ACTIVITY.**

Ganesh N. Alawandi¹, Kiran K. Pujar¹, Manohar V. Kulkarni^{1*}

¹P.G. Department of Chemistry, Karnatak University, Dharwad, Karnataka, India.

*Author for Correspondence: Manohar V. Kulkarni

P.G. Department of Chemistry, Karnatak University, Dharwad, Karnataka, India.

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ABSTRACT

An efficient and conventional method has been developed to synthesize mono- and dinitrated-4-bromomethyl coumarin scaffolds by using nitrating mixture. Nitration of 6-substituted-4-bromomethyl coumarins has resulted in 8-nitration whereas 7-substitution has lead to 6,8-dinitro coumarins. Chemical structures of the newly synthesized compounds were established by IR, NMR, mass spectral studies. All the title compounds were subjected to *in vitro* antibacterial testing against two pathogenic strains and antifungal screening against two fungi. Among the tested compounds, **2b** and **2c** showed significant antibacterial and antifungal activities. Also the compound **2d** and **2e** showed significant antifungal activity against *A. niger*.

KEYWORDS: Coumarin, Nitration, Antimicrobial.

INTRODUCTION

Coumarin derivatives have gained importance in medicinal chemistry in view of their structural diversity and biological activities.^[1-4] Presence of nitro group in chloramphenicol triggered off investigations on a variety of nitrogen heterocycles in general and also lead to the synthesis of coumarin analogues of chloramphenicol.^[5] Earlier studies focused on the influence of different groups on the formation of regioisomeric nitrocoumarins. *ortho*-Nitro derivatives of hydroxycoumarins have been found to exhibit anti-histamine^[6] and anti-cancer properties.^[7] Studies in this area have revealed the selective toxicity of trinitro-7-hydroxy coumarins towards human skin malignant melanocytes^[8] and their therapeutic role in the treatment of renal cell carcinoma.^[9] 6-Nitro coumarins have been useful precursors for 6-amino coumarins which have been employed in many functional group transformations

leading to bio-active compounds. Highly fluorescent aminocoumarins are used as optical brighteners and fluorescent probes.^[10] It is pertinent to mention that mononitration of hydroxycoumarins has been effectively achieved by using Thallium nitrate,^[11] ceric ammonium nitrate,^[12] and nitric oxide gas.^[13] Nitration of coumarins is greatly influenced by the substituents at C6, C7 and C4-positions. Under controlled conditions nitration of 7-methyl-4-bromomethyl coumarins afforded 6,8-dinitro compound the structure of which has been confirmed by X-ray analysis,^[14] where as nitration of 6-substituted-4-aryloxymethyl coumarins was accompanied by cleavage of the ether linkage leading to 5,8-dinitro-4-hydroxymethyl coumarins.^[15] **Figure-1** displays some of the nitroaromatic antibiotics produced by bacteria of the genus *Streptomyces* and **Figure-2** displays some of the biologically active nitro-coumarins.

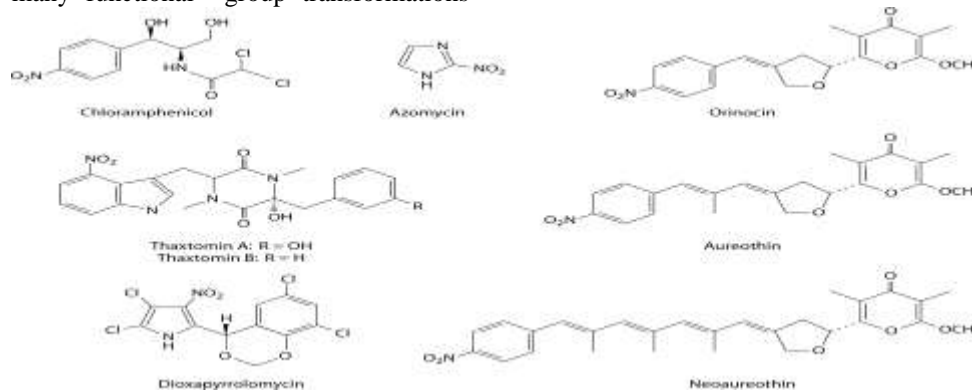


Figure-1. Nitroaromatic antibiotics produced by bacteria of the genus *Streptomyces*

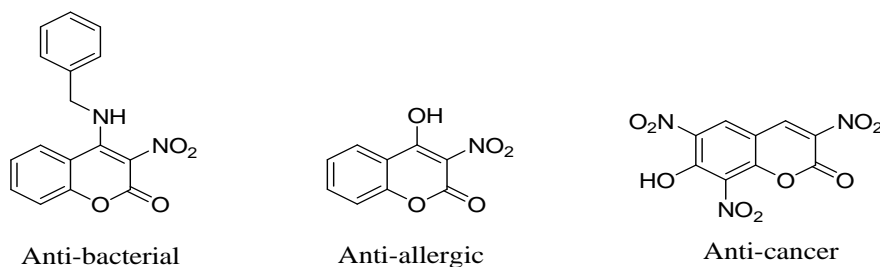
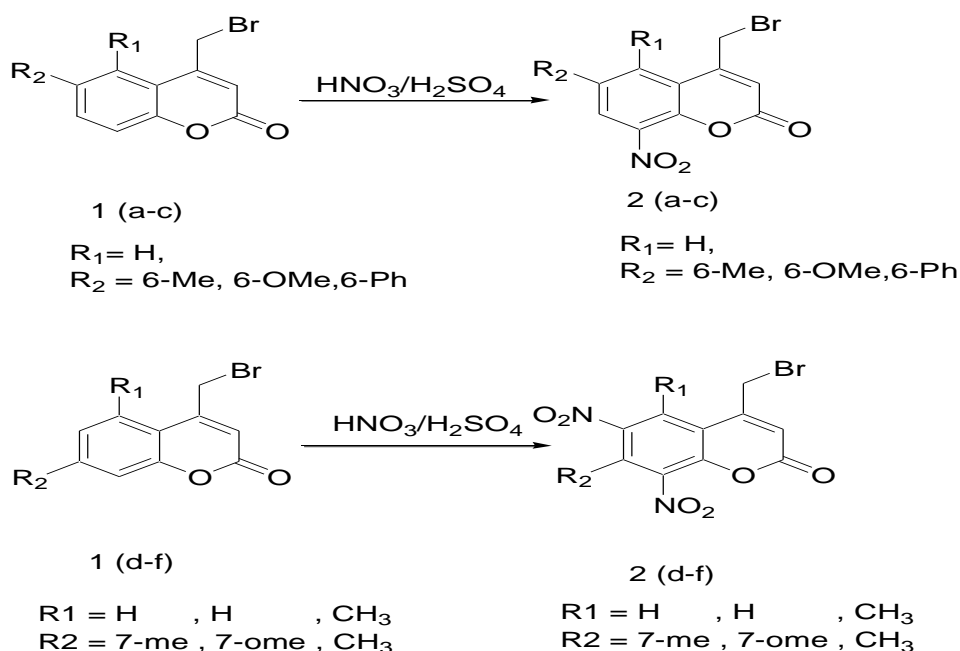


Figure -2: Biologically active nitrocoumarins

In the light of the above cited literature the interesting aspects associated with nitration of coumarins and biological importance of 4-hydroxy-nitro coumarins it was thought of considerable relevance to investigate nitration of 4-bromomethyl coumarins which is represented in Scheme 1.



Scheme 1. Mono and dinitration of 4-bromomethyl coumarins

Results and Discussion

The IR spectrum of 6-methyl-8-nitro-4-bromomethyl coumarin (**2a**) exhibited lactone carbonyl of coumarin at 1765 cm^{-1} and asymmetric and symmetric stretching vibration of NO_2 observed at 1539 cm^{-1} and 1350 cm^{-1} respectively, whereas $=\text{C-H}$, $\text{C}=\text{C}$ and C-O-C were observed at 3076 cm^{-1} , 1623 cm^{-1} and 1142 cm^{-1} respectively. The $^1\text{H NMR}$ spectrum of the same compound displayed two singlets at $2.57\text{ }\delta$ ppm, and $4.68\text{ }\delta$ ppm due to 6-CH_3 and $\text{C}_4\text{-CH}_2$ of coumarin whereas $\text{C}_3\text{-H}$ found to resonate at $6.64\text{ }\delta$ ppm and $\text{C}_5\text{-H}$ and $\text{C}_7\text{-H}$ at $8.34\text{ }\delta$ ppm.

The IR spectrum of 7-methyl-6,8-dinitro-4-bromomethyl coumarin (**2d**), exhibited lactone carbonyl of coumarin at 1743 cm^{-1} and asymmetric and symmetric stretching vibration of NO_2 observed at 1530 cm^{-1} and 1355 cm^{-1} , whereas stretching vibrations of $=\text{C-H}$, $\text{C}=\text{C}$ and C-O-C were observed at 3076 cm^{-1} , 1623 cm^{-1} and 1166 cm^{-1}

respectively. $^1\text{H NMR}$ spectrum of 7-methyl-6,8-dinitro-4-bromomethyl coumarin (**2d**) displayed singlet at $2.63\text{ }\delta$ ppm due to 7-CH_3 of coumarin and another singlet at $4.52\text{ }\delta$ ppm due to $\text{C}_4\text{-CH}_2$ of coumarin whereas $\text{C}_3\text{-H}$ found to resonate at $6.70\text{ }\delta$ ppm and $\text{C}_5\text{-H}$ at $8.49\text{ }\delta$ ppm. All the compounds **2(a-f)** were screened for their antimicrobial activity (**Table - 1**).

EXPERIMENTAL

Instrumentation

Melting points were determined with open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison, WI, USA) as KBr discs. $^1\text{H NMR}$ spectra were recorded on Bruker 400 MHz Spectrometer using CDCl_3 as solvents and TMS as internal standard. All chemical shifts were reported as δ values (ppm). Mass spectra were recorded using Shimadzu GCMSQP2010S. The elemental analyses were

carried out using Hereaus CHN rapid analyser. Purity of the compound was checked by TLC.

General Procedure for the Preparation of compounds 1(a-f)

To a mixture of equimolar quantities of substituted phenols (0.1 mol) and 4-bromoethylacetoacetate (0.1 mol) was added, 30 ml of conc. sulphuric acid with stirring and maintaining the temperature between 0-5°C. The reaction mixture was allowed to stand in ice chest for overnight and the deep-red coloured solution was poured in to crushed ice. The separated solid was filtered and washed with water and then with cold ethanol, so as to get a colourless compound. All the substituted bromomethyl coumarins **1(a-f)** were recrystallised from acetic acid.

General Procedure for the Preparation of compounds 2(a-f)

4-Bromomethyl coumarin 1(a-f) (2 g) was dissolved in conc. sulphuric acid (4 ml) and treated with nitrating mixture (2 ml Conc. HNO₃ and 4 ml Conc. H₂SO₄) at a temperature below 5°C. The resulting yellow colored liquid was kept overnight at room temperature. The reaction mixture was decomposed with crushed ice. Separated solid was filtered and washed with excess of water dried and all the nitro derivatives **2(a-f)** were recrystallised from acetic acid.

Spectral Data

4-Bromomethyl-6-methyl-8-nitro-chromen-2-one (2a).

Yellow solid; yield 70%; m.p 173 °C; IR (KBr) cm⁻¹ 1765 (C=O), 1539, 1350 (NO₂); ¹H NMR (DMSO, 400 MHz, TMS): δ 2.57 (s, 3H, C6-CH₃), 4.68 (s, 2H, C4-CH₂), 6.64 (s, 1H, C3-H), 8.34 (s, 1H, C8-H), 8.32 (s, 1H, C5-H). GC/MS *m/z*: 298 observed. Anal.Calcd. for C₁₁H₈BrNO₄; C,44.32; H, 2.71; N,4.70; Found: C,44.35; H,2.74; N,4.75.

4-Bromomethyl-6-methoxy-8-nitro-chromen-2-one (2b).

Yellow solid; yield 63%; m.p 184 °C; IR (KBr) cm⁻¹ 1762 (C=O), 1457, 1314 (NO₂); ¹H NMR (DMSO, 400 MHz, TMS): δ 3.58(s, 3H, C6-OCH₃), 4.89 (s, 2H, C4-CH₂), 7.2 (s, 1H, C3-H), 8.36 (s, 1H, C8-H), 8.33 (s, 1H, C5-H). GC/MS *m/z*: 314 observed. Anal.Calcd. for C₁₁H₈BrNO₅; C,42.06; H, 2.57; N,4.46; Found: C,42.10; H,2.61; N,4.51.

4-Bromomethyl-6-phenyl-8-nitro-chromen-2-one (2c).

Yellow solid; yield 68%; m.p 192 °C; IR (KBr) cm⁻¹ 1758 (C=O), 1452, 1334 (NO₂); ¹H NMR (DMSO, 400 MHz, TMS): δ, 4.82 (s, 2H, C4-CH₂), 7.32 (s, 1H, C3-H), 8.34 (s, 1H, C7-H), 8.32 (s, 1H, C5-H), 7.8-8.31(m, 5H, Ar-H) . GC/MS *m/z*: 360 observed. Anal.Calcd. for C₁₆H₁₀BrNO₄; C,53.36; H, 2.80; N,3.89; Found: C,53.39; H,2.85; N,3.94.

4-Bromomethyl-7-methyl- 6, 8-dinitro-chromen-2-one (2d).

Yellow solid; yield 65%; m.p 183°C; IR (KBr) cm⁻¹ 1743 (C=O), 1530, 1355 (NO₂); ¹H NMR (DMSO, 400 MHz, TMS): δ 2.63(s, 3H, C7-CH₃), 4.52 (s, 2H, C4-CH₂), 8.49 (s, 1H, C5-H), 6.70(s, 1H, C3-H). GCMS *m/z* 343 observed. Anal. Calcd. for C₁₁H₇BrN₂O₆; C,38.51; H,2.06; N,8.17; Found: C, 32.58; H,2.12; N, 8.23.

4-Bromomethyl-7-methoxy- 6, 8-dinitro-chromen-2-one (2e).

Yellow solid; yield 61%; m.p 237°C; IR (KBr) cm⁻¹ 1738 (C=O), 1452, 1337 (NO₂); ¹H NMR (DMSO, 400 MHz, TMS): δ 3.53(s, 3H, C7-OCH₃), 4.55 (s, 2H, C4-CH₂), 8.51 (s, 1H, C5-H), 6.73 (s, 1H, C3-H). GCMS *m/z* 359 observed. Anal. Calcd. for C₁₁H₇BrN₂O₇; C,36.79; H,1.96; N,7.80; Found: C,36.84; H,2.03; N,7.88.

4-Bromomethyl-5,7-dimethyl-6, 8-dinitro-chromen-2-one (2f).

Yellow solid; yield 57%; m.p 164°C; IR (KBr) cm⁻¹ 1737 (C=O), 1432, 1327 (NO₂); ¹H NMR (DMSO, 400 MHz, TMS): δ 2.36(s, 3H, C5-CH₃), 2.76(s, 3H, C7-CH₃) 4.59 (s, 2H, C4-CH₂), 6.60(s, 1H, C3-H). GCMS *m/z* 357 observed. Anal. Calcd. for C₁₂H₉BrN₂O₆; C,40.36; H,2.54; N,7.84; Found: C,40.41; H,2.59.; N,7.89.

Antimicrobial activity

The agar disc-diffusion method [16] was used for the screening of *in vitro* antimicrobial activity. The antimicrobial activity of the synthesized compounds **2(a-f)** were screened against *Staphylococcus aureus* and *Escherichia Coli* using nutrient agar medium. The antifungal activity of the compounds was tested against *Candia albicans* and *Aspergillus niger* using Sabour dextrose agar medium. The minimum inhibitory concentration (MIC) as carried out using micro dilution susceptibility method [17]. Ciprofloxacin was used as a standard antibacterial drug and Flucanazole was used as a standard antifungal drug. The observed data on the antimicrobial activity of compounds and control drugs are given in **Table-1**. The investigation of antibacterial screening **Table-1** revealed that some of the newly synthesized compounds showed moderate to good inhibition at 13-100 µg/mL in DMSO. Amongst all the compounds, compounds **2b**, **2c** showed excellent antibacterial activity against *E-Coli* (MIC: 13µg/mL) and *S .aureus* (MIC: 13 µg/mL). Compounds **2d** were displayed good activity against *S. aureus* (MIC: 25µg/mL). Compounds **2a** and **2e** exhibited moderate activity against *E. coli*. The investigation of antifungal screening **Table-1** revealed that some of the newly synthesized compounds showed moderate to good inhibition at 13-100 µg/mL in DMSO. Amongst the tested compounds, compounds **2b** and **2c** shows excellent inhibitory growth against *C. albicans* (MIC: 13 µg/mL) and *A niger* (MIC: 13µg/mL) respectively. Compound **2d** exhibited good activity against *A. niger* (MIC: 25µg/m). Remaining compounds showed moderate to less activity against both bacteria and fungi.

Table-1: Antimicrobial activity of compounds 2(a-f).

Compound	Bacterial strains (Gram +Ve and -Ve) in µg/mL		Fungal strains In µg/mL	
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. niger</i>
2a	100	50	75	75
2b	13	13	13	13
2c	13	13	13	13
2d	25	100	50	25
2e	100	50	50	25
2f	50	50	100	50
Ciprofloxacin	3.25	3.25	-	-
Flucanazole	-	-	3.25	3.25

CONCLUSION

In conclusion, we have described an efficient and conventional method for the synthesis of mono and dinitrated 4-bromomethyl coumarin scaffolds by using nitrating mixture. Nitration of 6-Substituted 4-bromomethyl coumarins has resulted in 8-nitration whereas 7-substitution has lead to 6,8-dinitro coumarins. All the title compounds were subjected to *in vitro* antibacterial testing against two pathogenic strains and antifungal screening against two fungi. Among the tested compounds, **2b** and **2c** showed significant antibacterial and antifungal activities with MIC 13µg/mL. Also the compound **2d** and **2e** showed significant antifungal activity against *A. niger* with MIC 25µg/mL. Further, Understanding the mechanism of biological action is still required in order to develop fully these compounds as potent antimicrobial drugs.

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