AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Synthesis, Characterization and Biological Evaluation of Some 1,3,4-oxadiazole Incorporated Coumarin Derivatives as Antifungal Agents

Muhammad Abul Kashem Liton*, Md. Sajid Hasan, Sabrina Helen, Mukta Das, Md. Shalauddin

Department of Chemistry, Faculty of Science, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh.

ABSTRACT

Coumarins are the family of benzopyrones, in which benzene ring amalgamated with the pyrone ring. Their versatile oxygen-containing heterocyclic structure and physicochemical characteristics are responsible to attract great attention from medicinal chemists and pharmacologists and for being a privileged scaffold in medicinal chemistry. In this study, 1, 3, 4 -oxadiazole incorporated a series of new coumarin derivatives have been synthesized. Structures of the newly synthesized compounds were established on the basis of FT-IR, ^{1}H NMR spectroscopic techniques and elemental analysis. The synthesized compounds were screened for their *in vitro* antifungal activity against five pathogenic fungal strains: *Aspergillus niger*, *Penicillium notatum*, *Saccharomyces cerevisiae*, *Penicillium chrysogenum* and *Neurospora crassa* by disk-diffusion method. All the compounds were found to have significant antifungal activity against all the fungal strains.

Keywords: Coumarins, 1,3,4-oxadiazole, Antifungal Activity.

*Corresponding Author Email: aklitonchemmbstu@gmail.com
Received 10 February 2022, Accepted 22 March 2022

Please cite this article as: Liton MA *et al*., Synthesis, Characterization and Biological Evaluation of Some 1,3,4-oxadiazole Incorporated Coumarin Derivatives as Antifungal Agents. American Journal of PharmTech Research 2022.

INTRODUCTION

Coumarins (Chromen-2-ones) are a group of naturally occurring benzopyrone framework containing organic compound [1]. Due to their inimitable and versatile oxygen-containing heterocyclic structure synthesis of coumarin and its derivatives have drawn great attention from organic and medicinal chemists for many years [2,3]. The large-conjugated system with electron-rich and charge-transport properties of this family of compounds, makes them attractive molecules for different fields of research [2]. Therefore, many coumarin derivatives have been extracted from natural sources, designed, synthesized, and evaluated on different pharmacological targets [4]. In addition, based on coumarin nucleus ion receptors, fluorescent probes, and biological stains are developed rapidly and forms widespread applications to guide timely enzyme activity, complex biological events, as well as accurate pharmacological and pharmacokinetic properties in living cells [5].

As an important group of organic heterocycles, coumarin derivatives possess many important biological activities such as antifungal [6], antibacterial [7], anti-inflammatory [8], antiproliferative [9], antitumor [10], antiviral [11], antioxidant [12], anticoagulant [13], anticancer [14] and anti-HIV [15] activities. Moreover, their unparalleled photochemical and physicochemical properties make them efficient in a diverse of applications such as optical brighteners [16], dispersed fluorescent and laser dyes [17], additives in food [18], perfumes [19], cosmetics [20], and pharmaceuticals [21]. So, the synthesis of coumarin derivatives have attracted great interest recently.

Our interest in synthesizing some advanced coumarin derivatives have been developed by seeing its widespread applications. So, in this study, we synthesized some 1, 3, 4-oxadiazole incorporated coumarin derivatives. All the synthesized compounds were fully characterized by spectroscopic techniques and elemental analysis and were screened for antifungal activity.

MATERIALS AND METHOD

All the chemicals were collected from Sigma-Aldrich and used without further purification. All the solvents were distilled using standard methods before use. The progress of the reaction and the purity of the synthesized compounds were determined by TLC using aluminum sheets of merck silica gel 60 F254 of 0.25 mm thickness detected by UV light (254 nm). Melting points were determined in open capillaries on Stuart SMP10 melting point apparatus and are uncorrected. The IR spectra (KBr discs) were recorded on a Perkin-Elmer FT-IR Spectrometer from thesis laboratory, Department of Chemistry, Mawlana Bhashani Science and Technology University (Bangladesh). The ^{1}H NMR spectra were recorded on BRUKER FT-NMR-AV400N spectrophotometer-300 MHz (Pharmaceutical Science Laboratory, Tokushima University, Japan)

and BRUKER AVANCE III – 400 MHz NMR instrument (Wazed Miah Science Research Centre, Jahangirnagar University, Bangladesh) in Chloroform-d, Methanol-d_4 and DMSO-d_6 solvent, using TMS as an internal reference and values of chemical shift were expressed in ppm. Elemental analysis was performed on PerkinElmer, ChemBioDraw Ultra, version 14.0.0.117. For the antifungal activity evaluation, MacConky agar, MHB (Mueller Hinton Brooth), PDA (Potato Dextrose Agar) were purchased from Oxoid, UK, and Econazole was purchased from Biomaxima, Poland, EU.

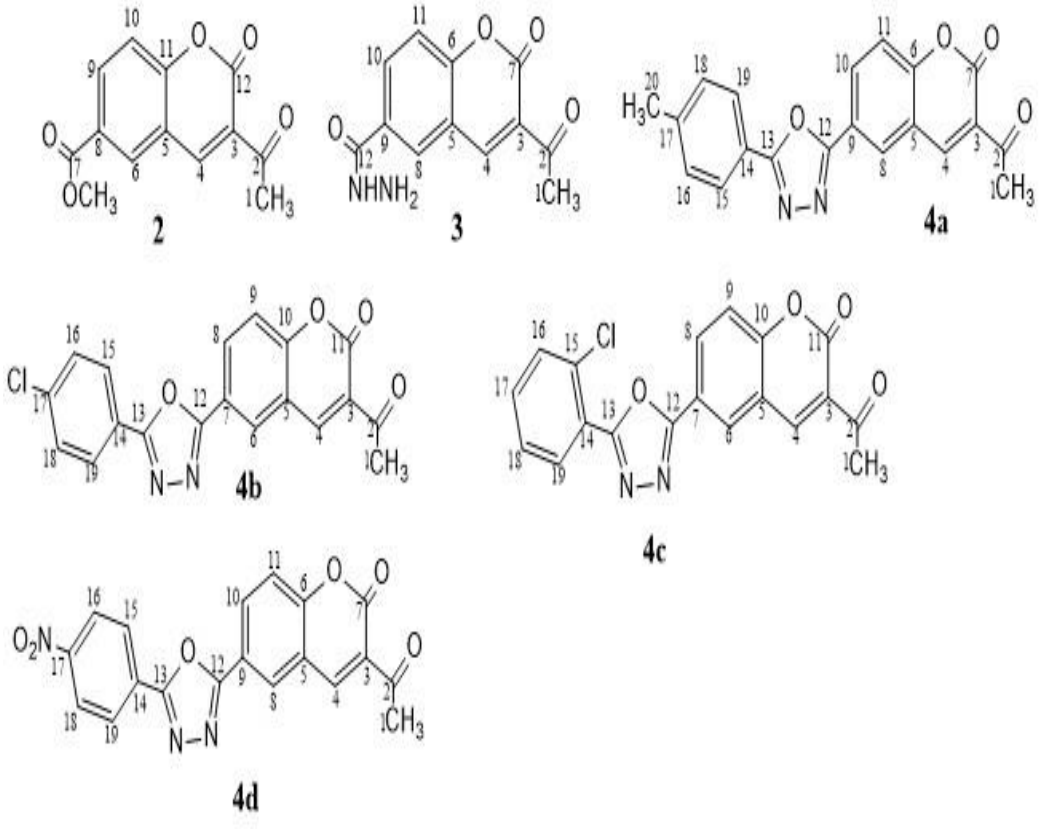


Figure 1: Structure of the synthesized compounds (Numbering was used for ^{1}H NMR assignment).

General procedures of synthesis and spectral data analysis

Synthesis of Methyl 3-acetyl-2-oxo-2H-chromene-6-carboxylate (2)

Methyl 3-acetyl-2-oxo-2H-chromene-6-carboxylate (**2**) was synthesized according to the Knoevenagel condensation reaction. To a solution of Methyl 3-formyl-4-hydroxybenzoate **1** (0.01 mole) and ethyl acetoacetate (0.011 mole) in ethanol (40 mL), few drops of piperidine was added. This mixture was stirred well for 2-3 hr. at room temperature. Progress of the reaction was monitored by TLC. After completion of the reaction, precipitate was filtered, dried and recrystallized from ethanol.

Methyl 3-acetyl-2-oxo-2H-chromene-6-carboxylate (2)

White crystal, Yield 84%, m.p. 207-209 °C, R_f 0.74 (Ethyl acetate- Petroleum ether = 2:8), FT-IR (KBr, cm^{-1}): ν = 3116, 3046 (Ar, C–H, str.), 1743 (C=O, lactone), 1716 (C=O, ester), 1684 (C=O, acetyl), 1619 (C=C, pyrone), 1292, 1237 (C-O, lactone); ^{1}H NMR (300 MHz, Chloroform-d): δ= 2.75 (s, 3H, CH_3, acetyl), 3.99 (s, 3H, OCH_3, ester), 7.45 (d, J = 6.0 Hz, H-10, Ar-H), 8.33 (d, J = 6.0, H-9, Ar-H), 8.34 (s, H-6, Ar-H) 8.56 (s, H-4, Py-H) ppm; Anal. Calcd. for $C_{13}H_{10}O_5$ (246.21): C 63.42, H 4.09, O 32.49; found: C 63.36, H 4.06, O 32.49.

Synthesis of 3-Acetyl-2-oxo-2H-chromene-6-carbohydrazide (3)

A mixture of Methyl 3-acetyl-2-oxo-2*H*-chromene-6-carboxylate **2** (0.001 mole) and hydrazine hydrate (0.004 mole) in absolute methanol (10-15mL) was refluxed on water-bath for 1-1.5 hr. at 64 °C temperature. The progress of the reaction was judged by TLC. After completion of the reaction, reaction-mixture was poured into ice cold water. The obtained precipitate thus filtered off, washed with water and dried. Finally, the product was purified by recrystallization from ethanol to give the compound **3**.

3-Acetyl-2-oxo-2H-chromene-6-carbohydrazide (3)

White powder, Yield 65%, m.p. 160-162 °C, R_f 0.70 (Ethyl acetate- Petroleum ether = 3:7), FT-IR (KBr, cm^{-1}): ν = 3373, 3309 (N–H, str., 1^o), 3231 (N–H, str., 2^o), 3000 (Ar, C–H str.), 1702 (C=O, lactone), 1601 (C=O, acetyl), 1582 (C=C, pyrone), 1495 (N-H, bend); ^{1}H NMR (300 MHz, Chloroform-d): δ = 2.75 (s, 3H, CH_3, acetyl), 5.54 (s, 2H, NH_2), 7.86 (d, J = 3.0 Hz, H-11, Ar-H), 7.90 (d, J = 3.0 Hz, H-10, Ar-H), 7.92 (s, H-8, Ar-H), 8.53 (s, H-4, py-H), 11.62 (s, 1H, NH) ppm; Anal. Calcd. for $C_{12}H_{10}N_2O_4$ (246.21): C 58.54, H 4.09, N 11.38, O 25.99; found: C 58.48, H 4.06, N 11.37, O 25.99.

Synthesis of 3-Acetyl-6-(substituted 5-phenyl-1, 3, 4-oxadiazol-2-yl)-2H-chromen-2-one derivatives (4a-4d)

A mixture of 3-Acetyl-2-oxo-2H-chromene-6-carbohydrazide **3** (0.001 mole), substituted benzoic acid (0.001 mole) and $POCl_3$ (0.11 mole) was refluxed on water-bath for 1-2 hr at 60-70 ^oC temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, resulting reaction mixture was cooled to room temperature and poured into crushed ice. Then the resulting solution was neutralized with 10 % $NaHCO_3$ solution. Then colored solid product was isolated by filtration and dried. Finally, purification by recrystallization with appropriate solvents gave desired products.

3-Acetyl-6-[5-(4-methylphenyl)-1, 3, 4-oxadiazol-2-yl]-2H-chromen-2-one (4a)

Yellow solid, Yield 68 %, m.p. 135-137 ˚C, R_f: 0.60 (Ethyl acetate- Hexane = 3:7), FT-IR (KBr, cm^{-1}): ν = 3070 (Ar, C–H, str.), 1725 (C=O, lactone), 1679 (C=O, acetyl), 1633, 1610 (C=N,

oxadiazole), 1578 (C=C, pyrone); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.42 (s, 3H, CH_3), 2.75 (s, 3H, CH_3, acetyl), 7.30 (d, J = 6.0 Hz, H-11, Ar-H), 7.44 (dd, J = 6.0 Hz, H-16, H-17, H-18, Ar-H), 7.83 (d, J= 6.0 Hz, H-10, Ar-H), 8.02 (d, J = 6.0 Hz, H-15, H-19, Ar-H), 8.44 (s, H-4, py-H) ppm; Anal. Calcd. for $C_{20}H_{14}N_2O_4$ (346.33): C 69.36, H 4.07, N 8.09, O 18.48; found: C 69.29, H 4.04, N 8.08, O 18.47.

3-Acetyl-6-[5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-yl]-2H-chromen-2-one (4b)

Pale yellow solid, Yield 72 %, m.p. 260-262 ˚C, R_f: 0.65 (Ethyl acetate - Hexane = 4:6), FT-IR (KBr, cm^{-1}): ν = 3034 (Ar, C–H, str.), 1694 (C=O, lactone), 1671 (C=O, acetyl), 1595 (C=N, oxadiazole), 771 (Ar-Cl); ^{1}H-NMR (300 MHz, Methanol-d_4): δ = 2.75 (s, 3H, CH_3, acetyl), 7.35 (dd, J = 6.0 Hz, H-8, H-9, Ar-H), 7.65 (d, J = 9 Hz, H-15, H-19, Ar-H) 7.92 (d, J = 6.0 Hz, H-16, H-18, Ar-H), 8.17 (s, H-6, Ar-H), 8.53 (s, H-4, py-H) ppm; Anal. Calcd. for $C_{19}H_{11}N_2O_4Cl$ (366.75): C 62.22, H 3.02, Cl 9.67, N 7.64, O 17.45; found: C 62.16, H 2.99, Cl 9.67, N 7.63, O 17.45.

3-Acetyl-6-[5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (4c)

Pale yellow solid, Yield 65 %, m.p. < 25 ˚C, R_f: 0.55 (Ethyl acetate - Hexane = 5:5); FT-IR (KBr, cm^{-1}): ν = 3015 (Ar, C–H, str.), 1718 (C=O, lactone), 1661 (C=O, acetyl), 1614 (C=N, oxadiazole), 1586 (C=C, pyrone), 771 (Ar-Cl); ^{1}H NMR (300 MHz, Chloroform-d): δ = 2.75 (s, 3H, CH_3, acetyl), 7.28-7.46 (m, H-17, H-18, Ar-H), 7.48 (d, J = 6.0 Hz, H-19, Ar-H), 7.60 (d, J = 6.0 Hz, H-9, Ar-H), 7.61 (d, J = 6.0 Hz, H-16, Ar-H), 8.13 (d, J = 6.0 Hz, H-8, Ar-H), 8.42 (s, H-4, py-H) ppm; Anal. Calcd. for $C_{19}H_{11}N_2O_4Cl$ (366.75): C 62.22, H 3.02, Cl 9.67, N 7.64, O 17.45; found: C 62.16, H 2.99, Cl 9.67, N 7.63, O 17.45.

3-Acetyl-6-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (4d)

Yellow solid, Yield 71 %, m.p. 202-204 ˚C, R_f 0.45; (Ethyl acetate - Hexane = 4:6), FT-IR (KBr, cm^{-1}): ν = 3114, 3081 (Ar, C–H, str.), 1694 (C=O, lactone), 1628 (C=O, acetyl), 1605 (C=N, oxadiazole), 1586 (C=C, pyrone); ^{1}H NMR (400 MHz, Chloroform-d): δ = 2.75 (s, 3H, CH_3, acetyl), 7.54 (d, J = 8 Hz, H-11, Ar-H), 8.14 (d, J = 8.0 Hz, H-15, H- 19, Ar-H), 8.75 (s, H-4, py-H) ppm; Anal. Calcd. for $C_{19}H_{11}N_3O_6$ (377.30): C 60.48, H 2.94, N 11.14, O 25.44; found: C 60.42, H 2.91, N 11.13, O 25.44.

Evaluation of antifungal activity

Preparation of complexes

The stock solution of all synthesized compounds was prepared by dissolving the compound in a minimum amount of DMSO. The trial samples had been prepared on four different concentrations

such as 1 mg/mL, 0.75 mg/mL, 0.50 mg/mL, 0.25 mg/mL which are indicated as a, b, c, d respectively. DMSO indicates the solvent.

Microbial culture

All the synthesized compounds were screened *in vitro* for their antifungal activity against five pathogenic fungal strains: *Aspergillus niger*, *Penicillium notatum*, *Saccharomyces cerevisiae*, *Penicillium chrysogenum* and *Neurospora crassa*. The fungi were identified following growth on appropriate media and morphological and microscopic characteristics [22].

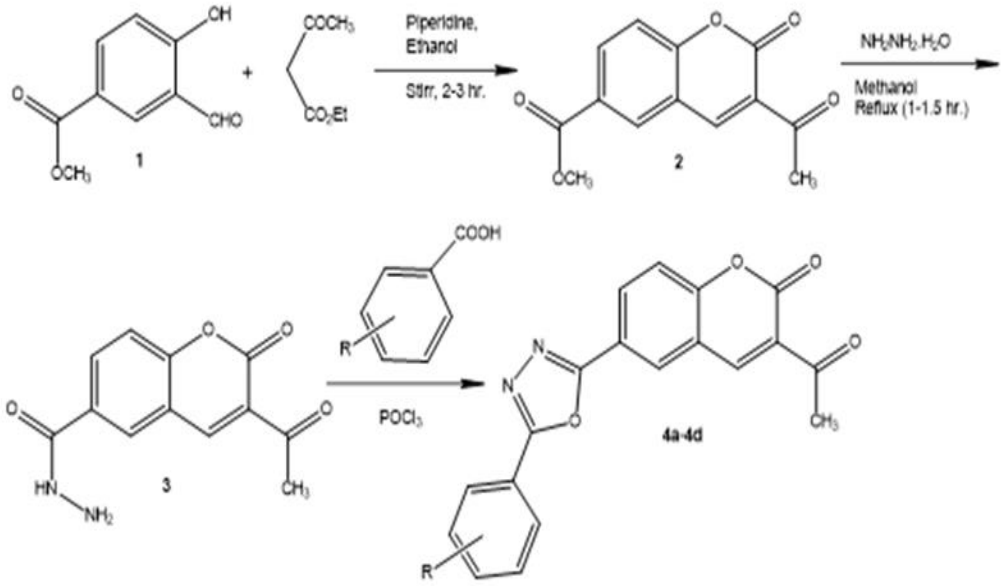
Antimicrobial Assay of the complexes

Antimicrobial assay of the complexes was performed by agar well diffusion method in Potato Dextrose Agar (PDA) plates for fungi. The synthesized complexes were considered as experimental compounds and solvent of the complexes was used as negative control. Econazole was used as positive control for fungi. For the experimental compounds, 20 micro-liter of liquid solution of each sample were soaked in filter paper disk. All discs were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface by maintaining sterile condition. It was allowed to diffuse for about 30 minutes at room temperature, incubated for 48-72 hours at 26 ºC for bacteria and fungi respectively. After incubation, plates were observed for the formation of a clear zone around the disks which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. in diameter.

RESULTS AND DISCUSSION

Chemistry

Coumarin derivative Methyl 3-acetyl-2-oxo-2H-chromene-6-carboxylate **2** was synthesized from Methyl 3-formyl-4-hydroxybenzoate **1** which combined with ethyl acetoacetate in the presence of piperidine in ethanol at room temperature in 84 % yields via Knoevenagel condensation [23]. Later on, treatment of Methyl 3-acetyl-2-oxo-2*H*-chromene-6-carboxylate 2 with hydrazine hydrate in methanol at 64 °C gave 3-Acetyl-2-oxo-2H-chromene-6-carbo- hydrazide 3 in 65 % yields [24]. Finally, treatment of various benzoic acid derivatives with 3-Acetyl-2-oxo-2H-chromene-6-carbohydrazide 3 in phosphorus oxychloride at 60-70 °C followed by neutralization with 10 % $NaHCO_3$ solution afforded new coumarin derivatives 4a-4d with 60–75 % yields (scheme-1)[25-26]. Structures of the newly synthesized compounds have been established on the basis of FT-IR, ^1H NMR spectroscopic techniques and elemental analysis.



4a. R = *p*-CH_3, **4b.** R = *p*-Cl, **4c.** R = *o*-Cl, **4d.** R = *p*-NO_2.

Scheme 1: Synthetic route of coumarin derivatives (4a-4d)

Antifungal Activity

The synthesized complexes (2, 3, 4a, 4b, 4c and 4d) exhibited a varying degree of inhibitory effects on the growth of different tested fungi strains. The solvent DMSO had no antifungal effect at the concentrations employed. The results were compared with Econazole used as standard. The activity of the complexes was recorded as zone of inhibition, ranged from 8 mm to 12 mm in diameter Figure 2.

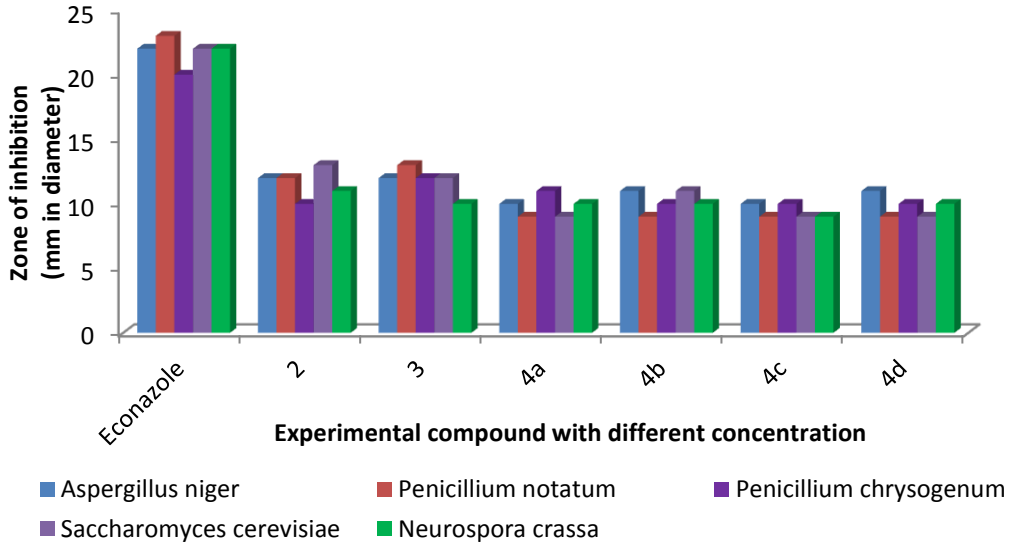


Figure 2: Antifungal activity of complexes against *Aspergillus niger, Penicillium notatum, Saccharomyces cerevisiae, Penicillium chrysogenum* and *Neurospora crassa fungal species.*

However, the results reveal that compounds **2, 3, 4a, 4b, 4c, and 4d** showed excellent antifungal activities against all the fungal strains. So, we may conclude that all the tested compounds have antifungal activities.

CONCLUSION

In the last few years, coumarin and coumarin related compounds turn out most promising field of research due to its unique and versatile oxygen-containing heterocyclic structure and its large biological, photochemical and therapeutically applications. In this study, some 1,3,4-oxadiazole incorporated coumarin derivatives have been synthesized and characterized by FT-IR, ^{1}H NMR spectroscopic techniques and elemental analysis. The in vitro antifungal screening of the synthesized compounds against five pathogenic fungal strains: *Aspergillus niger, Penicillium notatum, Saccharomyces cerevisiae, Penicillium chrysogenum and Neurospora crassa* by disk-diffusion method were carried out. All the compounds were found to have significant antifungal activity against all the fungal strains.

AUTHOR CONTRIBUTION STATEMENT

Muhammad Abul Kashem Liton: Conceptualized and designed the experiments. Md. Sajid Hasan: Performed the experiments in laboratory. Sabrina Helen: Analyzed and interpreted the data; wrote, revised and edited the paper. Mukta Das: Analyzed and interpreted the data. Md. Shalauddin: Evaluated the antifungal activity and interpreted the data.

REFERENCES

1. Zaffer AM, Ali M, Mir RS. New sesqui-and diterpenic coumarin ethers from the roots of aegle marmelos (L.). Corr. Nat. Prod. J. 2012; 2: 252-258.
2. Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. Curr. Med. Chem. 2005; 12: 887–916.
3. Rajitha B, Kumar VN, Someshwar P, Madhav JV, Reddy PN, Reddy YT. Dipyridine copper chloride catalyzed coumarin synthesis via Pechmann condensation under conventional heating and microwave irradiation. ARKIVOCXII 2006; 23–27.
4. Stefanachi A, Leonetti F, Pisani L, Catto M, Carotti A. Coumarin: A natural, privileged and versatile scaffold for bioactive compounds. Molecules 2018; 23: 250.
5. Carneiro A, Matos MJ, Uriarte E, Santana L. Trending topics on coumarin and its derivatives in 2020. Molecules 2021; 26: 501.

6. Nagamallu R, Srinivasan B, Ningappa MB, Kariyappa AK. Synthesis of novel coumarin appended bis (formyl pyrazole) derivatives: studies on their antimicrobial and antioxidant activities. Bioorg. Med. Chem. Lett. 2016; 26: 690–694.
7. Tanitame A, Oyamada Y, Ofuji K, Fujimoto M, Iwai N, Hiyama Y et al. Synthesis and antibacterial activity of a novel series of potent DNA Gyrase inhibitors. Pyrazole derivatives. J. Med. Chem. 2004; 47: 3693–3696.
8. Ghate M, Kusanur RA, Kulkarni MV. Synthesis and in vivo analgesic and anti-inflammatory activity of some bi heterocyclic coumarin derivatives. Eur. J. Med. Chem. 2005; 40: 882–887.
9. Musaa AM, Khanb FO, Cooper wood JS. Synthesis and antiproliferative activity of coumarin-estrogen conjugates against breast cancer cell lines. Lett. Drug Des. Discov. 2009; 6: 133–138.
10. Kostova I, Momekov G. New zirconium (IV) complexes of coumarins with cytotoxic activity. Eur. J. Med. Chem. 2006; 41: 717–726.
11. Zavrnik D, Muratovi S, Makuc D, Plavec J, Cetina M, Nagl A. Benzylidene-bis-(4-hydroxycoumarin) and benzopyrano coumarin derivatives: synthesis, 1H/13C-NMR conformational and X-ray crystal structure studies and in vitro antiviral activity evaluations. Molecules 2011; 16: 6023–6040.
12. Parameswaran M, Kochupappy RT, Gopalakrishnan S. Synthesis of coumarin heterocyclic derivatives with antioxidant activity and in vitro cytotoxic activity against tumour cells. Acta Pharm. 2009; 59: 159–170.
13. Crowther MA, Ginsberg JS, Julian J, Denburg J, Hirsh J, Douketis J et al. A comparison of two intensities of Ampicillin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. New Engl. J. Med. 2003; 349: 1133–1138.
14. Aoife L, O'Kenedy R. Studies on coumarins and coumarin related compounds to determine their therapeutic role in the treatment of cancer. Curr. Pharm. Design 2004; 10: 3797–3811.
15. Zhao H, Neamati N, Hong H, Mazumder A, Wang S, Sunder S et al. Coumarin-based inhibitors of HIV Integrase. J. Med. Chem. 1997; 40: 242–249.
16. Keskin SS, Aslan N, Bayrakceken F. Optical properties and chemical behavior of laser dye coumarin-500 and the influence of atmospheric corona discharge. Spectrochem. Acta: A Biomol. Spectros. 2009; 72: 254–259.

17. Christie RM, Morgan KM, Islam MS. Molecular design and synthesis of aryl sulfonated coumarin fluorescent dyes and their application to textile. Dyes Pigments 2008; 76: 741–747.
18. Wang YH, Avula B, Nanayakkara NPD, Zhao J, Khan IA. Cassia cinnamon as a source of coumarin in cinnamon-flavored food and food supplements in the United States. J. Agric. Food Chem. 2013; 61: 4470–4476.
19. Floc'h F, Mauger F, Desmurs JR, Gard A. Coumarin in plant and fruits: implication in perfumery. Perfum. Flavor. 2002; 27: 32–36.
20. Stiefel C, Schubert T, Morlock GE. Bioprofiling of cosmetics with focus on streamlined coumarin analysis. ACS Omega 2017; 2: 5242–5250.
21. O'Kennedy R, Thornes RD. Coumarins: Biology, Applications and Mode of Action. John Wiley and Sons: Chichester; 1997.
22. Cheesbrough M. District laboratory practice in tropical countries. 2nd ed., update, Part-2, Cambridge University Press, Part-2; 2012.
23. Knoevenagel E. About the condensirende effect of organic bases. *Chem. Ber*. 1904; 37: 4461-4463.
24. Rama Ganesh CK, Yadav DB, Venkatesh KB. Synthesis and biological evaluation of some innovative coumarin derivatives containing thiazolidin-4-one rings. Indian J. Chem. 2010; 49: 1151-1154.
25. Abdo NYM, Kamel MM. Synthesis and anticancer evaluation of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and Mannich bases. Chem. Pharm. Bull. 2015; 63: 369-376.
26. Wu W, Chen Q, Tai A, Jiang G, Ouyang G. Synthesis and antiviral activity of 2-substituted methylthio-5-(4-amino-2-methylpyrimidin-5-y)-1,3,4-oxadiazle derivatives. Bioorg. Med. Chem. Lett. 2015; 25: 243-246.