Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (2):60-64
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation of *in-vitro* antimicrobial activity of some newly synthesized 7-hydroxy 4-methyl coumarin congeners

Sudhir Kumar P. *and Sahoo J.

Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India

---

ABSTRACT

*A series of substituted 8-arylazo 7-hydroxy 4-methyl coumarin (4a-4g) were synthesized and in-vitro evaluation for their preliminary antibacterial activities against E.coli, Staphylococcus aurous, Bacillus subtilis and Pseudomonas aeroginosa were carried out by agar-well diffusion method. Initially, the diazotized primary aryl amines, 3a-3g were synthesized and followed by coupling with 7-hydroxy 4-methyl coumarin. The synthesized compounds were structurally elucidated by UV, IR, ^{1}HNMR and Mass spectroscopy. These compounds mainly existed in azo and hydrazone tautomeric equilibrium. Solvatochromic behavior of the compounds was also investigated by studying their UV-visible spectra of different organic solvents. Zone of inhibition results revealed that the product **4g** exhibited greater antibacterial potential against all bacterial strains and other compounds (**4a-4f**) showed moderate activity and compared to standard antibiotic, Ampicillin. Finally, it was concluded that the compounds having coumarin nucleus bearing sulfonamide and -N=N- functional groups in same molecular structural frame, the compound 4g had tremendous zone of inhibition against Staphylococcus aurous and Bacillus subtilis while other compounds were responsible for moderate antibacterial activity due to structural presence of nitro, methoxy and methyl group in phenyl ring. The change in the maximum absorbance of the synthesized compounds in different solvents was determined and the solvatochromic property of the compound showed a medium red shift and showed moderate solvent dependency over the bathochromic shift.*

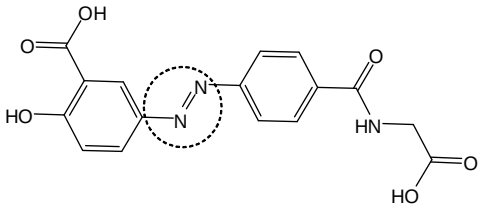
Keywords: 7-hydroxy 4-methyl coumarin (HMC); solvatochromic; sulfanilamide; diazotization; antibacterial activity

---

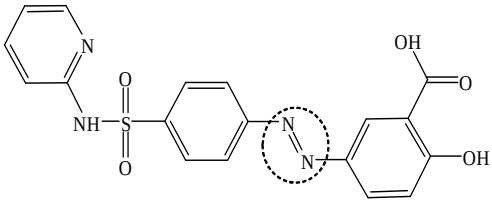
INTRODUCTION

Azo compounds are a very important class of chemical substances receiving much more attention in scientific research. Literature survey revealed that the azo dyes have been widely used in dying textile fibers, biomedical studies and advanced applications in organic synthesis as well as shows variety of interesting biological activities including antibacterial [1] and antifungal activities [2]. The synthetic heterocyclic azo compounds have been widely used in different pharmacological actions including antibacterial [3], antiviral [4], anti-fungal [5] and antioxidant activities [6]. It has been already reported that the medicinal properties of azo compounds particularly prepared from various organic compounds viz. acetyl salicylic acid, thymol, para-amino salicylate and β-napthol *etc*. Depending on the substituted in the ring system of 7-hydroxy 4-methyl coumarin derivatives have been a huge range of biological activities including antioxidant, antimicrobial, antiviral, anhelminthetic antitumor and anticoagulant activity. In addition to this application 7-hydroxy 4-methyl coumarin bearing azo moiety was extensively used as synthetic dyes and no antimicrobial evaluation has been reported. So, the inadequate of biological information could be traced on the synthesis of azo derived candidate substituting at C-8 position of 7-hydroxy 4-methyl coumarin moiety. In view of such biological reports, the present research work is aim to design and develop the synthesized of some new potential 7-hydroxy 4-methyl coumarin candidates bearing azo moiety and explored their *in-vitro* antibacterial

action. A series of arylazo compounds incorporating at C-8 of 7-hydroxy 4-methyl coumarin moiety by azocoupling reaction. Some of medicinal interest compounds containing azo moiety as follows



Basalazide (anti-inflammatory)



Sulfasalazine (anti-ulcerative colitis)

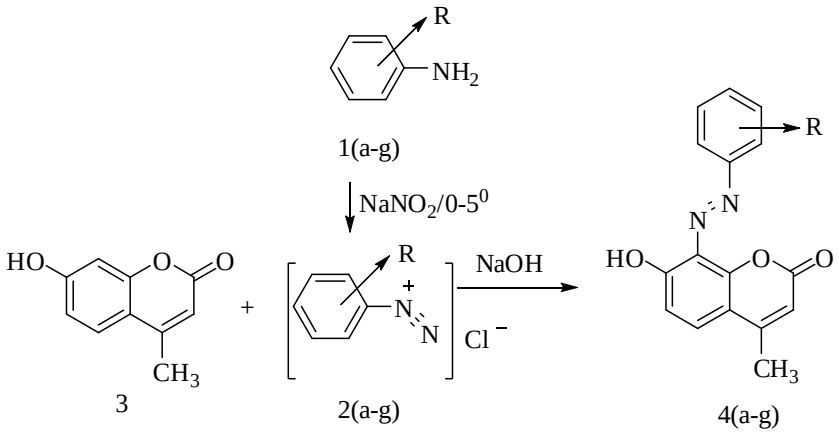
MATERIALS AND METHODS

Instruments and methods

The chemicals used in the present studies are of synthetic grade, Merck company Ltd. The M.Ps. were determined by open capillary method and uncorrected. The products were characterized by IR (JASCO FT/IR 4100 Spectrophotometer using KBr disc), ^{1}HNMR (Bruker ^{1}HNMR 400MHZ) using TMS as an internal standard), UV (JASCO V-630 Spectrophotometer), LC-MS (Shimadzu-Mass spectrophotometer) and elemental analysis was carried out by using Perkins Elmer-2400 C H N/ S Analyser system.

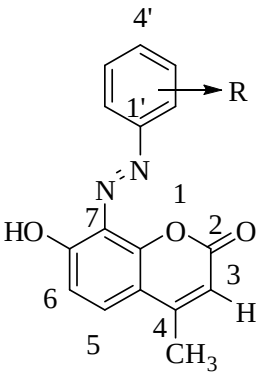
Synthesis of substituted 8-arylazo 7-hydroxy 4-methyl coumarin [7]

To a stirred suspension of sulfanilamide/aromatic amine (0.87mmole) in water (3ml), conc. HCl (3ml) was added with heating until complete dissolution and was cooled at o^{0}C on ice bath. To this mixture, $NaNO_2$ (0.9mmole, 60mg) dissolved in a minimum amount of water was slowly added with stirring for 15 mins. Then, it has been added to an ice cold solution of 7-hydroxy 4-methyl coumarin (HMC) (0.9mmole) and sodium hydroxide in ethanol (20ml). The reaction mixture was allowed to stir at (0-5^{0}C) for one hr and then the solid was collected by filtration. The crude products thus obtained, were dried and recrystallised from ethanol to give corresponding compounds (4a-4g).



R= 4-SO_3H, 4-NO_2, 3-NO_2, 4-CH_3, 2- CH_3, 4-OCH_3, 4-SO_2NH_2

Characterization of newly synthesized (4a-4g) compounds



(E)-4-((7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl) diazenyl) benzenesulfonic acid (4a)
Yield: 75%, M.p >300 ^{o}C, Rf 0.7, Brick red, IR (KBr) cm^{-1} 3250 (OHstr.), 3030 (Ar-H), 1698 (C=O str. of lactone), 1491(-N=N-), 1621(C=C str. coumarin), 1350, 1155 (SO_2 str. sulfonic acid), 832(Ar-CH def); ^{1}HNMR ($CDCl_3$) δ: 6.35(s, 1H, coumarin H-3), 2.45(s, 3H, CH_3), 7.87 (d, 1H, coumarin H-5), 7.25 (d, 1H, coumarin H-6), 7.65-8.22 (m, 4H, Ar-H); Analysis calcd for $C_{16}H_{12}N_2O_3$: C, 53.33; H, 3.36; N, 7.77, S, 8.90 Found: C 53.32; H 3.43; N 7.76, S, 8.85.

(E)-7-hydroxy-4-methyl-8-((4-nitrophenyl) diazenyl)-2H-chromen-2-one (4b)
Yield: 85%, M.p 255-260^{o}C, Rf 0.6, yellow, IR (KBr) cm^{-1} 3225 (OH str.), 3030 (Ar-H), 1710 (C=O str. of lactone), 1460 (-N=N-), 1621(C=C str. coumarin), 1550, 1360 (NO_2 str.), 835(Ar-CH def); ^{1}HNMR ($CDCl_3$) δ:9.65 (s, 1H, OH), 6.24 (s, 1H, coumarin H-3), 2.42(s, 3H, CH_3), 7.83(d, 1H, coumarin H-5), 7.12(d, 1H, coumarin H-6), 7.65-8.42(m, 4H, Ar-H); Analysis calcd for $C_{16}H_{11}N_3O_5$: C, 59.08; H, 3.41; N, 12.92;Found: C 59.05; H 3.39; N 12.82; m/z: 325.07 (100.0%), 326.07 (18.6%).

(E)-7-hydroxy-4-methyl-8-((3-nitrophenyl) diazenyl)-2H-chromen-2-one (4c)
Yield: 65%, M.p 265-270^{o}C, Rf 0.7, yellow, IR (KBr) cm^{-1} 3230 (OH str.), 2985 (Ar-H), 1720 (C=O str. of lactone), 1455(-N=N-), 1626(C=C str. coumarin), 1545, 1340 (NO_2 str.), 855(Ar-CH def); ^{1}HNMR ($CDCl_3$) δ:9.65(s, 1H, O<u>H</u>), 6.24 (s, 1H, coumarin H-3), 2.42(s, 3H, CH_3), 7.83(d, 1H, coumarin H-5), 7.12(d, 1H, coumarin H-6), 7.65-8.42(m, 4H, Ar-H); Analysis calcd for $C_{16}H_{11}N_3O_5$: C, 59.08; H, 3.41; N, 12.92; Found: C 59.02; H 3.37; N 12.90; m/z: 325.07 (100.0%), 326.07 (18.6%).

(E)-7-hydroxy-4-methyl-8-(p-tolyldiazenyl)-2H-chromen-2-one (4d)
Yield: 65%, M.p 280-290^{o}C, Rf 0.7, red, IR (KBr) cm^{-1} 3230 (OH str.),2885 (Ar-CH_3), 3020 (Ar-H), 1715 (C=O str. of lactone), 1445 (-N=N-), 1620 (C=C str. coumarin), 835(Ar-CH def); ^{1}HNMR ($CDCl_3$) δ:9.82 (s,1H,O<u>H</u>), 6.22(s, 1H, coumarin H-3), 2.32 (s, 3H, CH_3), 2.47(s, 3H, CH_3), 7.87(d, 1H, coumarin H-5), 7.11(d, 1H, coumarin H-6), 7.45-8.56(m, 4H, Ar-H); Analysis calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; Found: C 69.35; H 4.75; N 9.45; m/z: 294.10 (100.0%), 295.10 (19.2%), 296.11 (1.6%).

(E)-7-hydroxy-4-methyl-8-(o-tolyldiazenyl)-2H-chromen-2-one (4e)
Yield: 65%, M.p 255-260^{o}C, Rf 0.7, red, IR (KBr) cm^{-1} 3230 (OH str.),2955 (Ar-CH_3), 3020 (Ar-H), 1725 (C=O str. of lactone), 1460 (-N=N-), 1620 (C=C str. coumarin), 755(Ar-CH def); ^{1}HNMR ($CDCl_3$) δ:9.56 (s, 1H, O<u>H</u>), 6.23(s, 1H, coumarin H-3), 2.25 (s, 3H, C<u>H</u>$_3$), 2.47(s, 3H, C<u>H</u>$_3$), 7.96(d, 1H, coumarin H-5), 7.05(d, 1H, coumarin H-6), 7.09-7.88 (m, 4H, Ar-H); Analysis calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; Found: C 69.35; H 4.75; N 9.45; m/z: 294.10 (100.0%), 295.10 (19.2%).

(E)-7-hydroxy-8-((4-methoxyphenyl) diazenyl)-4-methyl-2H-chromen-2-one (4f)
Yield: 85%, M.p 265-270^{o}C, Rf 0.8, red, IR (KBr) cm^{-1} 3330 (OH str.),2865 (Ar-OCH_3), 3050 (Ar-H), 1720 (C=O str. of lactone), 1485 (-N=N-), 1620 (C=C str. coumarin), 835(Ar-CH def); ^{1}HNMR ($CDCl_3$) δ:9.56 (s,1H,O<u>H</u>), 6.23(s, 1H, coumarin H-3), 2.25 (s,3H.C<u>H</u>$_3$), 2.47(s, 3H, C<u>H</u>$_3$), 7.96(d, 1H, coumarin H-5), 7.05(d, 1H, H-6), 7.09-7.88 (m, 4H, Ar-H); Analysis calcd for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03; Found: C 68.75; H 4.53; N 9.05 m/z: 310.10 (100.0%), 311.10 (18.7%), 312.10 (2.6%).

(E)-4-((7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl) diazenyl) benzene sulfonamide (4g)
Yield: 85%, M.p 285-295^{o}C, Rf 0.7, Ruby red, IR (KBr) cm^{-1} 3340 (OH str.), 3050 (Ar-H), 1715 (C=O str. of lactone), 1455 (-N=N-), 1620 (C=C str. coumarin), 1365, 1170 (SO_2 str. sulfonamide), 840 (Ar-CH def); ^{1}HNMR ($CDCl_3$) δ:10.45 (s,1H,O<u>H</u>),7.39 (s, 2H, SO_2N<u>H</u>$_2$), 2.52(s, 3H, C<u>H</u>$_3$), 6.23(s, 1H, coumarin H-3), 7.96(d, 1H, coumarin H-5), 7.05(d, 1H, coumarin H-6), 8.06-8.44 (m, 4H, Ar-H); Analysis calcd for $C_{16}H_{13}N_3O_5S_4$: C, 53.48; H, 3.65; N, 11.63; Found: C 53.45; H 3.53; N 11.65 m/z: 359.06 (100.0%), 360.06 (18.4%), 361.05 (4.5%).

Antibacterial activity by Agar-well diffusion method
Antibacterial activity of the synthesized compounds was evaluated by agar-well diffusion method [8]. One strain from each bacterial species was used for monitoring antibacterial activities of the compounds. Bacterial lawn was prepared on 6 mm thick agar layer and kept for 30 min in BOD incubator as described in the earlier reported method. The wells (6 mm depth) were prepared with the help of sterilized cork borer and each well was based by 50 µL molten MH agars. Further, wells were filled with 100 µL aliquots of 100µg/mL synthesized compound. Plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition. Ampicillin 100µg/mL was used as reference controls, for all the synthesized compounds. The synthesized compounds causing the zone of inhibition of 20 mm or more were considered highly active and that having a zone of inhibition less than 14 mm was considered moderately active.

RESULTS AND DISCUSSION

A series of 8-arylazo 7-hydroxy 4-methyl coumarin (4a-4g) were synthesized by coupling of diazonium salt of sulfanilamide/Aryl primary amine derivatives 3(a-g) with 7-hydroxy 4-methyl coumarin in presence of NaOH. The precursor of HMC was synthesized by using mixture of resorcinol and ethylacetoacetate in presence PPA. In this reaction initially generate electrophilic diazonium salt N_2^+ from aryl primary amines and then coupled with reactive side of HMC to obtain desired above candidates. In all the synthesized compounds the following peaks were confirmed by FT/IR, those peaks range at 3218-3180, 1480-1455, 1610-1600 and 1720-1698 cm^{-1} were assigned the functional groups of –OH str., –N=N-, -C=C-aromatic str. and C=O str. of α-pyrone of coumarin respectively. The signal of amine group was not observed in the ^{1}HNMR spectral data and also not showed the absorption band of amine was in FTIR spectral data in all the synthetic dyes 4a-4g. The compound 4g, showed IR peaks at 1365 and 1170 cm^{-1} due to the presence of SO_2 str. of SO_2NH_2 and in 4c showed IR peak at 1360 and 1554cm^{-1} due to the presence of NO_2 str. The NH proton of the SO_2NH_2 group was observed ^{1}HNMR at δ7.39 ppm as singlet in compound **4g**.

Solvatochromic study of newly synthesized compounds

The solvent effects of the products were studied on UV-Visible spectrophotometer. The absorption spectra of these dyes 4a-4g were recorded in different solvents at a concentration of 10^{-5} to 10^{-6} M, and results are summarized in Table-2. According to UV experimental data in Table-2, the synthesized dye 4b shows bathochromic shift in 1, 4-dioxane and THF with respect to the λ_{max} as compared to the other polar solvents. These bathochromic shifts can be attributed to the interaction H-atom of amino proton of dyes with polar aprotic solvent such as THF because of increase polarity of the dyes system, generally in the excited state. Introduction of 4-nitrophenylazo substituent into the 7-hydroxy 4-methyl coumarin at the C-8 position gives largest bathochromic shift compare to other aryl azo dyes in all the solvents used. Other hand the presence of 3-nitro phenylazo 4c at the C-8 position gives rise to more bathochromic shift, when compare to 4-methoxy phenylazo 4f in using solvent such as THF.

Antibacterial activity evaluation

The *in vitro* antimicrobial activities of all the synthesized compounds (**4a-4g**) were evaluated against gram positive (*S. aureus* sub-culture and *B. subtilis* sub-culture) and gram negative bacteria (*E.coli* MTCC 614 and *P. aeruginosa* MTCC 1035). The activity potentials were qualitatively assessed by the presence or absence of zone of inhibition. The results given in Table 1 showed that the compound **4g** (E)-4-((7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl) diazenyl) benzene sulfonamide have greater potential as an antimicrobial agent against *S. aureus*, *B.subtilis*, *E.coli* and *P. aeruginosa* than other compounds. The maximum inhibition zones produced by compound **4g** against *S.aureus*, *B.subtilis*, *E.coli and P. aeruginosa* were 20, 23, 20 and 15 mm respectively. The other synthesized compounds (**4a-4f**) have been exhibited significant antibacterial activity against *S.aureus*, *B.subtilis*, *E.coli* and *P. aeruginosa*. Ampicillin was used as positive control due to it is commonly used antibiotic against gram positive and some of gram negative pathogenic bacterial species. The structural presence of sulphamido, -N=N- and coumarin in same structural frame in all synthesized compounds may be responsible for antibacterial activity, which is in agreement with the previous reports.

Table-1. Antimicrobial properties of the synthesized compound expressed as Zone of inhibition (mm)

Compd.	Microorganisms & Zone of Inhibition (mm)			
(conc.100 µg/ml)	*E.coli*	*S.aureus*	*B.subtilis*	*P.aeruginosa*
4a	18	16	17	13
4b	17	15	15	14
4c	15	16	15	15
4d	14	17	16	12
4e	17	19	17	13
4f	16	18	18	14
4g	20	23	20	15
Ampicillin	23	24	25	18

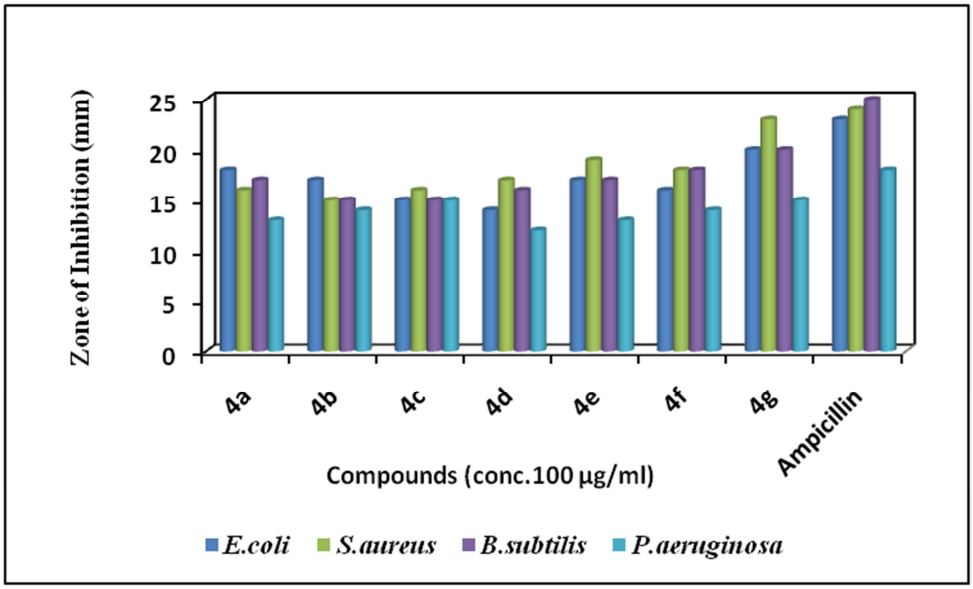


Fig.1 Antimicrobial activity of azo derived 7-hydroxy 4-methyl coumarin analogues (4a-4g)

Table-2. UV-Visible spectral data (λ_{max}, nm) of newly synthesized Coumarin analogues (4a-4g) using different solvents

Compds.	λ_{max} (Methanol)	λ_{max} (Ethanol)	λ_{max} (Isopropanol)	λ_{max} (1,4Dioxane)	λ_{max} (THF)
4a	365	367	-	370	373
4b	397	398	398	435	440
4c	393	390	391	425	427
4d	-	385	-	385	387
4e	383	385	384	378	374
4f	390	388	387	395	398
4g	365	364	368	379	376

CONCLUSION

The present research work involves the synthesis of novel 8-arylazo 7-hydroxy 4-methyl coumarin candidates and to explore their antibacterial activity. All compounds have remarkable activity compared to Ampicillin. Hence, it is concluded that there is a scope of further study on developing some lead compounds for the treatment of chronic urinary tract bacterial infections.

Acknowledgements
Authors are grateful to Siksha 'O' Anusandhan University for financial and technical support. The authors are also thankful to the Director, NISER, Bhubaneswar, India for providing ^{1}HNMR of the synthesized compounds reported herein. They are again thankful to the Director of Piramal Healthcare, Ahmadabad for providing LC-MS data.

REFERENCES

[1] J W Daniel. *Toxicol Appl Pharmacol* **(1962)**; 4:572.
[2] Y Jinghua and Z Guanxing. *Fenxi Huaxue* **(1998)**; 26(12):1519.
[3] I M Awad, A A Aly, A M Abdel Alim, R A Abde, S H Ahmed. *J Inorg Biochem* **(1988)**; 33(2): 77-89.
[4] M Tonelli, I Vazzana, B Jasso, V Boida, F Sparatore, et al. *Biorg Med Chem* **(2009)**; 17: 4425-4440.
[5] K R Raghavendra, K Ajay Kumar. *Int J Chemtech Res* **(2013)**; 5(2): 1756-1760.
[6] A A H Kadham, A A Al-Amiery, AY Musa, A B Mohamad. *Int J Mol Sci* **(2011)**; 12: 5747-5761.
[7] J Sahoo, S K Mekap and P Sudhir Kumar. *J Taibah Univ Sci* **(2014)**;
http://dx.doi.org/doi:10.1016/j.jtusci.2014.08.001
[8] C Perez, M Pauli and P Bazerque. *Act Bio Med Exper* **(1990)**; 15: 113-115.