June 2009 *Volume 5 Issue 2*

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 5(2), 2009 [221-225]

Synthesis of some new coumarin derivatives with biological activity

Satish B.Jadhav*, Ranjana A.Shastri, Kishor V.Gaikwad

P.G. dept. of chemistry, Milind College of science Aurangabad-431001, Maharashtra, (INDIA)

E-mail : orgchem.jadhav@yahoo.co.in

Received: 23rd February, 2009 ; Accepted: 1st March, 2009

ABSTRACT

Two series of coumarin derivatives were synthesized by reacting respectively various 7-Hydroxy chromene-2-one 1a-d with 3-(2-chlorophenyl)-5-methyl isoxazole-4-carbonyl chloride and 3-(2, 4-dichlorophenyl)-5-methyl isoxazole-4-carbonyl chloride to afford a novel biologically more potent compound which is substituted 2-oxo-2H-chromen-7-yl-3-(2-chlorophenyl)-5-methyl isoxazole-4-carboxylate **2a-d** and various substituted 2-oxo-2H-chromen-7-yl-3-(2,4-dichlorophenyl)-5-methyl isoxazole-4-carboxylate **3a-d**. All the synthesized products are evaluated for their antibacterial activity against *Escherichia coli, Bacillus cirroflagellosus and Salmonella typhi and antifungal activity against Aspergillus niger, Rhizoctonia bataticola* and *Penicillium*. Characterisation of all the compounds has been done by IR, ^{1}H NMR, MS and elemental analysis. All the compounds exhibited significant to moderate antifungal and antibacterial activities.

KEYWORDS

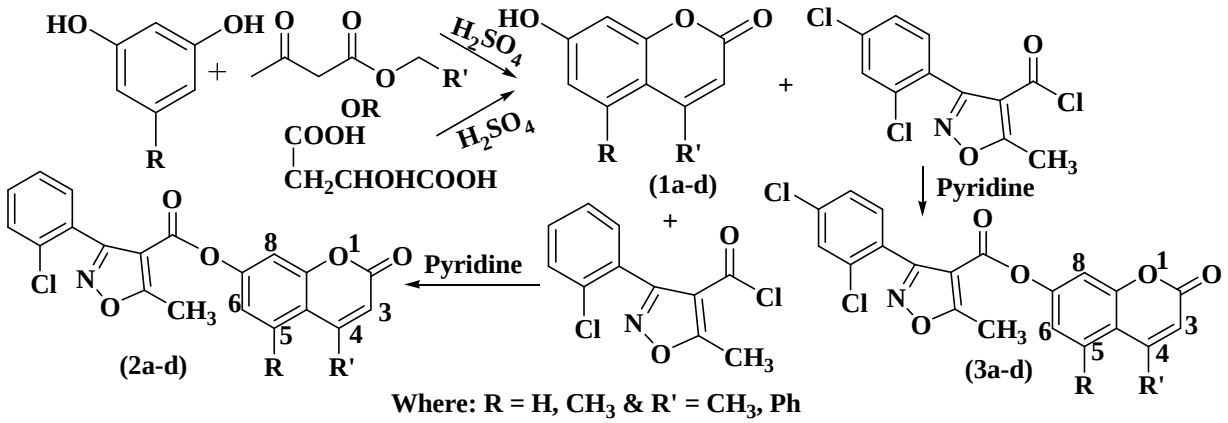
7-Hydroxy chromene-2-one;
Derivatives of coumarin;
Antimicrobial activity.

INTRODUCTION

Among a wide variety of heterocycles that have explored for developing pharmaceutically important molecules, coumarin and its derivatives represent one of the most active class of compounds possessing a wide spectrum of biological activity[1]. Many of the synthetic coumarin have provide to be diverse biological activities such as antitumor[2,3], antibacterial[4,5], antifungal [6-8], anti-coagulant[9], spasmolytic[10], anti-inflammatory[11], anthelmintic[12], diuretic[13]. In addition, some of the derivatives of coumarin are also used as additive to food and cosmetics[14]. Due to the interesting chemical and biological properties of coumarin and derivatives prompted their synthesis in order to study their biological and pharmacological activity. Five membered heterocycles like isoxazoles have found wide application as pharmaceutical and agrochemical agents. Compounds bearing isoxazole moiety consist of various biological applications such as antitumor[14], analgesic[16], antimicrobial[17] and chemotherapy[18].

Literature survey revealed that when one biodynamic heterocyclic system was coupled with another, a molecule with enhanced biological activity[19,20] was produced. The chemistry of these linked biheterocycles have been the fascinating field of investigation in medicinal chemistry as they have been found to exhibit enhanced biological profile[21]. In view of the biological and medicinal activity of coumarin and isoxazoles and in continuation of our work on biheterocycles compound[22], it was thought worth-while to synthesis and investigates the activity of the compounds in which coumarin moiety has been linked with isoxazole. We report in this paper, the synthesis and antimicrobial activity of various substituted 2-oxo-2H-chromen-7-yl-3-(2-chlorophenyl)-5-methyl isoxazole-4-carboxylate and various substi-

Full Paper



Where: R = H, CH_3 & R' = CH_3, Ph

SCHEME 1

TABLE 1 : Physical constant and spectral data of compounds (2a-d) and (3a-d)

Compd.	R	R'	mp ^{0}C	Yield%	IRcm^{-1}	^{1}H NMR δ
(2a)	H	H	199	85	3100(Ar-H), 2945(C-H from CH_3),1760(-O-C=O of ester), 1740(lactone C=O), 1612 (C=C),1625(C=N)	2.34(s, 3H, -CH_3), 6.34-7.24(m, 5H, coumarin), 7.36-7.42(m, 4H, Ar-H)
(2b)	H	CH_3	168	80	3180(Ar-H), 2950(C-H from CH_3),1765(-O-C=O of ester), 1735(lactone C=O), 1610 (C=C),1620(C=N)	2.34(s, 3H, 4-CH_3), 2.45(s, 3H, -CH_3), 6.12(s, 1H, 3-H),6.83-6.84(d, 1H, 8-H), 6.89-6.92(dd, 1H, 6-H), 7.38-7.44(m, 4H, Ar-H), 7.45(d, 1H, 5-H)
(2c)	CH_3	H	215	82	3110(Ar-H), 2955(C-H from CH_3),1750(-O-C=O of ester), 1735(lactone C=O), 1625 (C=C),1645(C=N)	2.33(s, 3H, 4-CH_3), 2.42(s, 3H, -CH_3), 6.11-6.13(d, 1H, 3-H),6.63(s, 1H, 6-H), 6.82(s, 1H, 8-H), 7.32(dd, 1H, 4-H), 7.43-7.47(m, 4H, Ar-H)
(2d)	H	Ph	160	75	3080(Ar-H), 2940(C-H from CH_3),1760(-O-C=O of ester), 1735(lactone C=O), 1620 (C=C),1640(C=N)	2.43(s, 3H, -CH_3), 6.13(s, 1H, 3-H), 6.82-6.84(d, 1H, 8-H),6.88-6.91(dd, 1H, 6-H), 7.12-7.30(m, 5H, Ar-H),7.37-7.45(m, 4H, Ar-H), 7.46-7.47(d, 1H, 5-H)
(3a)	H	H	211	80	3095(Ar-H), 2940(C-H from CH_3),1755(-O-C=O of ester), 1735(lactone C=O), 1617 (C=C),1625(C=N)	2.44(s, 3H, -CH_3), 6.36-7.27(m, 5H, coumarin),7.39-7.43(m, 2H, Ar-H), 7.45(s, 1H, Ar-H)
(3b)	H	CH_3	220	70	3100(Ar-H), 2965(C-H from CH_3),1765(-O-C=O of ester), 1730(lactone C=O), 1615 (C=C),1630 (C=N)	2.30(s, 3H, 4-CH_3), 2.42(s, 3H, -CH_3), 6.10(s, 1H, 3-H),6.80-6.82(d, 1H, 8-H), 6.85-6.88(dd, 1H, 6-H),7.35-7.38(m, 2H, Ar-H), 7.41(s, 1H, Ar-H), 7.45(d, 1H, 5-H)
(3c)	CH_3	H	163	82	3095(Ar-H), 2950(C-H from CH_3),1760(-O-C=O of ester), 1730(lactone C=O), 1612 (C=C),1635(C=N)	2.88(s, 3H, 4-CH_3), 2.42(s, 3H, -CH_3), 6.12-6.14(d, 1H, 3-H), 6.60(s, 1H, 6-H), 6.80(s, 1H, 8-H), 7.34(dd, 1H, 4-H), 7.36-7.39(m, 2H, Ar-H), 7.43(s, 1H, Ar-H)
(3d)	H	Ph	172	72	3100(Ar-H), 2945(C-H from CH_3),1755(-O-C=O of ester), 1735(lactone C=O), 1610(C=C),1625(C=N)	2.42(s, 3H, -CH_3), 6.11(s, 1H, 3-H), 6.83(s, 1H, 8-H),6.90-7.01(dd, 1H, 6-H), 7.10-7.28(m, 5H, Ar-H),7.32-7.34(m, 2H, Ar-H), 7.40(s, 1H, Ar-H),7.45-7.46(d, 1H, 5-H)

tuted 2-oxo-2H-chromen-7-yl-3-(2,4-dichlorophenyl)-5-methyl isoxazole-4-carboxylate, in which both coumarin and isoxazole is in one moiety.

RESULT AND DISCUSSION

In the present work, 7-hydroxy coumarin were synthesized by using Pechmann and Diusberg condensation method[23,24]. 7-hydroxy coumarin were prepared

TABLE 2 : Antibacterial screening results of the compounds (2a-d) and (3a-d)

Comp.	Antibacterial activity zone inhibition		
	E.coli	*B.cirroflagellosus*	*Salmonella typhi*
(2a)	16	15	17
(2b)	10	12	14
(2c)	12	09	13
(2d)	14	10	12
(3a)	15	14	10
(3b)	12	13	11
(3c)	10	10	12
(3d)	12	08	09
Norfloxacin	28	25	30
DMSO	-ve	-ve	-ve

-ve no antibacterial activity

TABLE 3 : Antifungal screening results of the compounds (2a-d) and (3a-d)

Comp.	Antifungal activity		
	A.niger	*R.bataticola*	*Penicillium*
(2a)	-ve	+ve	+ve
(2b)	-ve	-ve	-ve
(2c)	+ve	-ve	+ve
(2d)	+ve	+ve	+ve
(3a)	-ve	-ve	+ve
(3b)	+ve	-ve	-ve
(3c)	+ve	+ve	-ve
(3d0	+ve	+ve	+ve
Griseofulvin	-ve	-ve	-ve
Control -	+ve	+ve	+ve

+ve - Growth - - - No Antifungal activity, -ve - No growth- - - - Antifungal activity observed

by using substituted resorcinol and different condensing agents i.e. ethylacetoacetate, ethyl benzoylacetate and malic acid to obtained **(1a-d)**. Compounds **(1a-d)** was converted into sodium salt and then condensation with 3-(2-chlorophenyl)-5-methyl isoxazole-4-carbonyl chloride and 3-(2, 4-dichlorophenyl)-5-methyl isoxazole-4-carbonyl chloride in presence of pyridine yielded compounds **(2a-d)** and **(3a-d)** respectively (SCHEME 1). All the compounds where characterized by the analytical and spectroscopic methods (TABLE 1).

The IR spectrum of the compound 1b showed a sharp peak at 3500cm^{-1} assigned to the OH stretch. The peaks at 3015 and 2890 cm^{-1} were due to aromatic and aliphatic C-H stretch. A strong absorption at 1675cm^{-1} was assigned to C=O stretch. The peak at 1135cm^{-1} was attributed to lactone stretch. The ^{1}H NMR (δ, DMSO-d_6) spectrum of the compound 1b showed a broad singlet at 10.45 for OH proton, two sharp singlet at δ 6.03 and 2.30 for C_3H $4CH_3$ protons respectively. The aromatic protons at C_5 and C_8 appeared as doublets at δ 7.52 and 6.60 respectively and the protons at C_6 appeared at 6.8 as a doublet of doublet. The IR spectrum of **(2b)** and **(3b)** in KBr suggested the absence of OH group at 3500cm^{-1} and ^{1}H NMR (δ, DMSO-d_6) spectrum of the compound **(2b)** and **(3b)** showed absence of broad singlet at δ 10.45 it suggest that formation of product.

Antimicrobial activity

All the compounds **(2a-d)** and **(3a-d)** were screened for their antimicrobial activity in *vitro* at doses of 100μg in 0.1ml of DMF against the bacteria Escherichia coli, Bacillus cirroflagellosus and Salmonella typhi using norfloxacin as standard and for their antifungal activity in *vitro* against the fungi Aspergillus niger, Rhizoctonia bataticola and Penicillium using Griseofulvin as standard and DMF was used as culture medium and the method employed was cup-plate method[25,26]. The zone of inhibition was measured in mm and compared with standard drugs.

The investigation of antibacterial screening revealed that compounds **(2a**, **2d**, **3a)** are moderately active towards E. coli where as compounds **(2a**, **2b**, **3a**, **3b)** are moderately active towards B. cirroflagellosus, similarly compounds **(2a-c)** are moderately active towards Salmonella typhi. Rests of the compounds were weakly active towards all the bacteria. The investigation of antifungal activity data reveled that compounds **(2a**, **2b**, **3a)** shows inhibitory effect against A. niger and compounds **(2b**, **2c**, **3a**, **3b)** shows inhibitory effect against R. bataticola, similarly compounds **(2b**, **3b**, **3c)** shows inhibitory effect against Penicillium. Remaining compounds are inactive against all the fungus result shown in TABLES 2 and 3 respectively.

Experimental section

Melting points (m.p.) were determined in open capillary tube and are uncorrected. IR spectra were recorded using a Perkin-Elmer 1600FT spectrometer at a ca. 5-15% solution in DMSO-d_6 or $CDCl_3$ (TMS as internal standard), GCMS was recorded on Perkin-Elmer clarus 500 mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with ultraviolet lights (254nm).

Full Paper

Physical constants and spectral characterization data of all the compounds reported in this paper are summarized in TABLE 1.

Preparation of 7-hydroxy coumarin (1a)

A mixture of resorcinol (30g), malic acid (25g) and sulphuric acid (60ml) was heated on an oil bath at 120^{0}C until the effervescence ceased (about 2 hrs.). The reaction mixture was cooled and poured over excess of crushed ice. The precipitation solid was filtered and washed with cold water. It was crystallized from aqueous ethanol. M.P. 226

Preparation of 7-hydroxy 4-methyl coumarin (1b)

A solution resorcinol (55g) in ethyl acetoacetate (65ml) was added to concentrated sulphuric acid (500ml) dropwise with constant stirring at 0^{0}C. The reaction mixture was left overnight and poured over ice. The resulting solid was filtered, washed with water and crystallized from 60% ethanol. M.P. 185^{0}C

Preparation of 7-hydroxy 5-methyl coumarin (1c)

A mixture of orcinol (25g), malic acid (27g) and sulphuric acid (50ml) was refluxed on a water bath for 2hrs. The reaction mixture was cooled and poured over ice. The obtained solid was filtered and washed with water. It was crystallized from ethanol. M.P. 245^{0}C

Preparation of 7-hydroxy 4-phenyl coumarin (1d)

To an ice-cold mixture of resorcinol (22g) and ethyl benzoylacetate (38g), phosphoric acid (100ml) was added slowly. The reaction mixture was kept at room temperature for 24 hrs. It was then poured on ice. The precipitated solid was filtered and washed with water. It was crystallized from ethanol. M.P. 241^{0}C

Preparation of 4-methyl-2-oxo-2H-chromen-7-yl-3-(2-chlorophenyl)-5-methyl isoxazole-4-carboxylate (2b)

7-hydroxy 4-methyl coumarin (0.1M) was dissolved in pyridine (0.3M) to this 3-(2-chlorophenyl)-5-methyl isoxazole-4-carbonyl chloride (0.1M) was added with constant shaking. The reaction mixture was refluxed for 1hr. and then poured on crushed ice and the pyridine was neutralized by using dil. HCl to obtained crude product. The solid obtained was filtered, washed with water and crystallized from 75% alcohol.

Other compounds were prepared the similar way using substituted coumarin and 3-(2-chlorophenyl)-5-methyl isoxazole-4-carbonyl chloride. Characterization data are presented in TABLE 1.

Compound (2b): Yield 80%, m.p.168^{0}C, (Anal. Calcd for $C_{21}H_{14}ClNO_5$: C, 63.73; H, 3.57; N, 3.54 Found, C, 63.43; H, 3.18; N, 3.25%), ^{1}H NMR: 2.34(s, 3H, 4-CH_3), 2.45(s, 3H, -CH_3), 6.12(s, 1H, 3-H), 6.83-6.84(d, 1H, 8H), 6.89-6.92(dd, 1H, 6-H), 7.38-7.44(m, 4H, Ar-H), 7.45(d, 1H, 5-H)

Preparation of 4-(4, 5-dihydro-5-phenyl isoxazole-3-yl) phenyl-3-(2,4-dichlorophenyl)-5-methyl isoxazole-4-carboxylate (3b)

7-hydroxy 4-methyl coumarin (0.1M) was dissolved in pyridine (0.3M) to this 3-(2, 4-dichlorophenyl)-5-methyl isoxazole-4-carbonyl chloride (0.1M) was added with constant shaking. The reaction mixture was refluxed for 1hr. and then poured on crushed ice and the pyridine was neutralized by using dil. HCl to obtained crude product. The solid obtained was filtered, washed with water and crystallized from 75% alcohol.

Other compounds were prepared the similar way using substituted coumarin and 3-(2, 4-dichlorophenyl)-5-methyl isoxazole-4-carbonyl chloride. Characterization data are presented in TABLE 1.

Compound (3b): Yield 70%, m.p.220^{0}C, (Anal. Calcd for $C_{21}H_{13}Cl_2NO_5$: C, 58.62; H, 3.05; N, 3.26 Found, C, 58.13; H, 2.78; N, 2.95%), ^{1}H NMR: 2.30(s, 3H, 4-CH_3), 2.42(s, 3H, -CH_3), 6.10(s, 1H, 3-H), 6.80-6.82(d, 1H, 8H), 6.85-6.88(dd, 1H, 6-H), 7.35-7.38(m, 2H, Ar-H), 7.41(s, 1H, Ar-H), 7.45(d, 1H, 5-H).

ACKNOWLEDGMENTS

The authors are thankful to Mr. A.R.Muley dept. of chemistry, Miss V.G.Ambhore Dept. of Biotechnology Milind College of science Aurangabad for help in antimicrobial study. The authors are also thankful to the Principal Dr. B.C. Ghoble for providing facilities.

REFERENCES

[1] R.D.Murray, J.Mendez, S.A.Brown, R.Murray, J.Mendez, S.Brown; 'The Natural coumarins: Oc-

currence, Chemistry and Biochemistry', John Wiley and Sons, New York, NY, **227**, **(1982)**.
[2] L.Raev, E.Voinov, I.Ivanov, D.Popov; Antitumar activity of some coumarin derivatives, Pharmazie, **45**, 696 **(1990)**; Chem.Abstr., **114**, 74711B **(1990)**.
[3] Z.M.Nofal, M.El-Zahar; Molecule, **5**, 99-113 **(2000)**.
[4] A.M.El-Agrody, M.S.Abd El-Latif, N.A.El-Hady, A.H.Fakery, A.H.Bedair; Molecule, **6**, 519-527 **(2001)**.
[5] S.Pratibha, P.Shreeya; Indian J.Chemistry, **38B**, 1139-1142 **(1999)**.
[6] T.Patony, G.Y.Litkei, R.Bognar, J.Erdei, C.Misztic; Pharmazie, **39(2)**, 86-91 **(1984)**.
[7] R.M.Shaker; Pharmazie, **51**, 148 **(1996)**.
[8] A.F.El-Farargy; Egypt J.Pharm.Sci., **32**, 625 **(1991)**.
[9] D.Lednier, L.A.Mitscher; 'The Organic Chemistry of Drug Synthesis', John Wiley and Sons, New York, **330**, **(1977)**.
[10] L.L.Andreani, E.Lapi; Bull.Chem.Farm, **99**, 583 **(1960)**.
[11] M.V.Kulkarni, B.G.Pujar, V.D.Patil; Arch.Pharm., **316(1)**, 15 **(1981)**.
[12] Y.L.Zhang, B.Chen, K.Zheng, M.Xu, L.Zhang, X. Lei; Yaoxue XueBao, **17**, 17 **(1997)**; Chem.Abstr., **96**, 1982, 135383 **(1982)**.
[13] L.Bonsignore, G.Loy, D.Secci, L.Calignano; European J.Med.Chem., **28**, 517 **(1993)**.
[14] R.O.Kennedy, R.D.Thornes; Coumarins: Biology, Applications and Mode of Action, Wiley and Sons, **(1997)**.
[15] Getal; J.Antibiot, **28(1)**, 91 **(1975)**.
[16] H.Kano, I.Adachi, R.Kido, K.Hirose; J.Med. Chem., **10(3)**, 411, **(1967)**.
[17] P.B.Reddy, S.M Reddy, E.Rajanarendar, A.K. Murthy, Indian Phytopathology, **37**, 370 **(1984)**.
[18] A.Sadanandam, M.V.Rajam, K.Subash, E. Rajanarendar; Indian Bot.Report, **3(1)**, 38 **(1984)**.
[19] C.Boschail, A.Cana, R.Disfilo, A.Frutlero, Gasco; Bioorg.Med.Chem., **7**, 1727 **(2000)**.
[20] P.Moloney Gerand, Martin Graemar, Mathew; J.Chem.Soc.Perkin Trans, **19**, 2725 **(1999)**.
[21] R.D.Clark, J.M.Carron, A.F.Kloge, D.B.Repke, A.P. Roszkowski, A.M.Stosberg, S.B.Earkar, S.M.Bitter, M.D.Okando, J.Med.Chem., **26(50)**, 657 **(1983)**.
[22] S.D.Rathod, K.V.Gaikwad, S.V.Gaikwad, S.B. Jadhav; Organic Chemistry: An Indian Journal., **4**, 9-11 **(2008)**.
[23] Van Pechmann; H.Ber., **17**, 929 **(1884)**.
[24] S.Sethna, R.Phadke, R.Adams; 'Organic Reactions', John Wiley, London, **6**, 1 **(1953)**.
[25] F.Kavanagh; 'Analytical Microbiology', Academic Press, New York, 125 **(1963)**.
[26] H.W.Seeley, P.J.Van Denmark; Microbes in action: A laboratory Manual of Microbiology, D.B. Taraporevala Sons and Co.Pvt.Ltd., Bombay, 55-80 **(1975)**.