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SYNTHESIS AND ANTI-MICROBIAL ACTIVITY OF NOVEL COUMARINS CONTAINING SUNSTITUTED-1,3,4-OXADIAZOLE DERIVATIVES

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ABSTRACT

An efficient general method has been described for the synthesis of novel 4-methyl-7-(5-sunstituted-1,3,4-oxadiazol-2-yl)methoxy)-2*H*-chromen-2-ones derivatives **5(a-h).** Compound 7-hydroxy-4-methyl coumarin treated with α-bromo ethylacetate in the presence of potassium carbonate in acetone as solvent to give compound ethyl-2-(4-methyl-2-oxo-2*H*-chrome-7-yloxy)acetate **(2)**, followed by treated with hydrazine hydrate to form corresponding 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)aceto hydrazide **(3).** The compound **3** is condensed with substituted aromatic aldehydes than followed by cyclization with glacial acetic acid to yield the title compounds **5(a-h)**. These analogs were evaluated for their antimicrobial activity against *Staphylococcus aureus (*Gram positive bacteria) *Escherichia Coli* (Gram Negative bacteria) and *Aspergillus niger*, *Candida albicans* (fungi) The analogs **5e**, identified as potent activity and **5f**, **5g** showed moderate activity against antimicrobial agents. Structural elucidation of all the newly synthesized title compounds has been established by the spectroscopic data IR, ^{1}HNMR, mass and elemental analysis.

KEYWORDS: 7-hydroxy-4-methyl coumarin, 1,3,4-oxadiazole, antibacterial strains, antifungal strains.

INTRODUCTION

The five member ring heterocyclic compounds have been studied by many researchers because of chemical and variable biological effects.[1] 1,3,4 Oxadizoles represent an important class of heterocyclic compounds that have many applications in the daily life.[2]

The replacement of acid and ester functionality in medicinal chemistry continues to be a popular strategy in the search for compounds with superior pharmacokinetic profiles. In particular, 1,3,4-oxadiazole rings have been of interest to medicinal chemists for many years, because of their anti-microbial[3], anti-biotic,[4] anti-inflammatory,[5] anti-convulsant[6] and anti-hepatitis B[7] activities. In addition to their utility as bioactive molecules, 1,3,4-oxadiazoles are useful intermediates for organic synthesis.[8]

Organic compounds containing coumarin moiety (2*H*-1-Chromen-2-one) are widely distributed in nature. Coumarin and its derivatives have been essentially found in green plants belonging to the family of Rutaceae and Umbelifferae. Coumarins are important oxygen containing fused hetero-cycles used in drugs and dyes.[9] These are having wide spread of applications as HIV protease and bacteriostatic agents.[10-11] However, the most widely reported activities for coumarin derivatives are their anti-inflammatory and anti-cancer activities.[12-13] Natural coumarins are known to have anti diabetic activity[14], anabolic, anti-oxidant and hepato protective activities.[15] The incorporation 1,3,4-oxadiazoles group as a component into parent coumarin alters the property of parent coumarin converts it into a more useful product. Recently, these investigations have revealed their potentials as versatile bio-dynamics agent. Consequently, the synthesis of compounds containing this hetero-cycle core has attracted considerable attention and a wide variety of methods have been used for its assembly.

MATERIALS AND METHODS

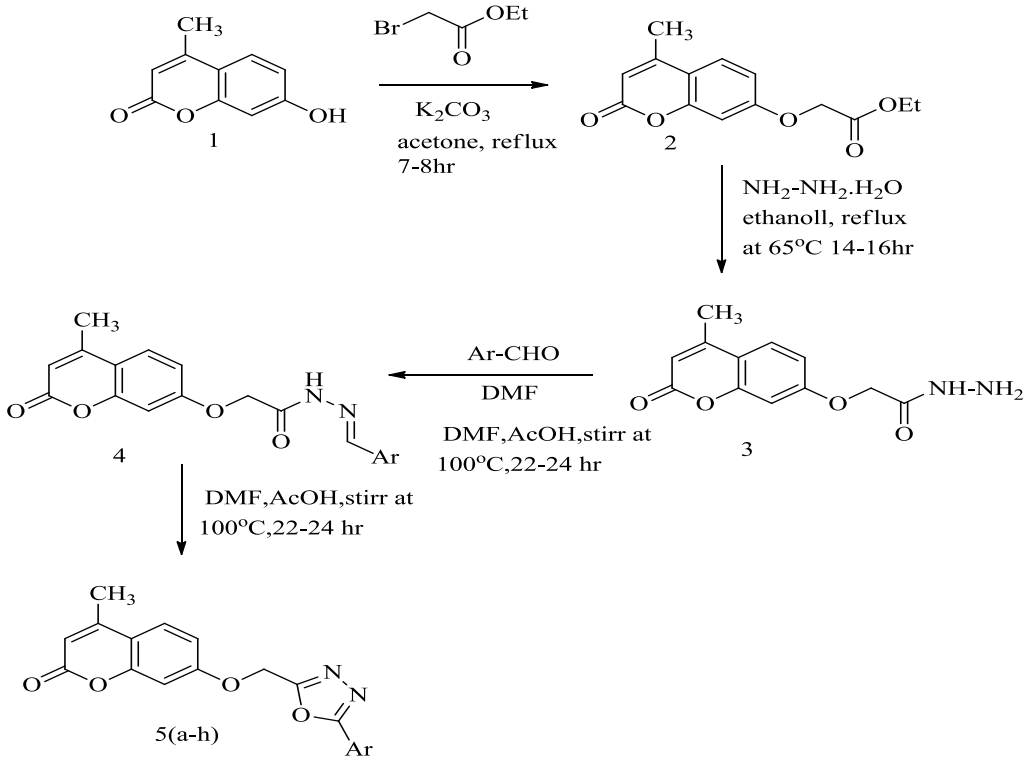
Melting points were determined in open capillaries and were uncorrected. Column chromatography was performed using silica-gel (100–200 mesh size) purchased from Thomas Baker, and thin-layer chromatography (TLC) was carried out using aluminium sheets pre-coated with silica gel 60F254 purchased from Merck. IR spectra (KBr pellets) were recorded on Shimadzu FT-IR model 8010 spectrophotometer. ^{1}H NMR spectra (DMSO-d_6) were taken a Varian mercury spectrometer (model YH- 300 FT NMR) using TMS as internal standard and chemical shift are expressed in δ ppm. Mass spectra were taken on Jeol sx-102/PA-6000 (EI) spectrometer.

RESULTS AND DISCUSSION

Compound 7-hydroxy-4-methyl coumarin **(1)** can be prepared by known process from resorcinol treated with ethylacetoacetate by Pechman condensation[16] method, which reacts with α-bromo ethylacetate in the presence of potassium carbonate in the presence of acetone

as solvent to give compound ethyl-2-(4-methyl-2-oxo-2*H*-chrome-7-yloxy)acetate **(2)**, followed by treated with hydrazine hydrate to form corresponding 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)aceto hydrazide **(3).** The compound **3** is condensed with substituted aromatic aldehydes than followed by cyclization with glacial acetic acid to yield the title compounds **5(a-h)**. The structure of the all newly synthesized compounds was elucidated on the basis of their spectral(IR, ^{1}HNMR, and mass) and elemental analyses data. The IR spectrum of 1632 (C=N), 1407 (N–N), 1247 (C–O–C) **5(a)** showed characteristic absorption bands. The ^{1}H NMR spectrum of compounds **5(a-h)** showed peaks at δ 7.41 to 8.34 ppm due to aromatic protons and exhibited two singlet signals at δ 2.45 ppm due to one methyl protons another singlet at δ 5.25-5.45 ppm for –CH_2 protons.

Scheme-I



S.No	Compound	Ar
1.	5a	Ar
2.	5b	2-OH
3.	5c	4-OH
4.	5d	4-OCH_3
5.	5e	4-Cl
6.	5f	4-NO_2
7.	5g	2-NO_2
8.	5h	2-CH_3

Ethyl-2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (2)

To a solution of 7-hydroxy-4-methyl-2*H*-chromen-2one (0.01mol), in acetone (10 mL), anhydrous pot. Carbonate (0.5gms) and α-bromoethyl acetate (0.01mol) was added slowly and refluxed for 7-8 hr. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, poured into ice cold water and extracted with chloroform (3x10 mL). The organic layers were collected, washed with brine solution (3x10 mL), dried over anhydrous Na_2SO_4 and concentrated under *vaccuo* to get corresponding compounds, than purified by re-crystallization with ethanol.

7-(2-hydrazinyloxy)-2-oxoethoxy)-4-methyl-2*H*-chromen-2-one (3)

To a solution of ethyl-2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy)acetate **(**0.01 mol), in 15 ml of absolute ethanol and added hydrazine hydrate (0.01mol). The resultant reaction mixture was refluxed at 65^{o}C for 14-16hrs. After completion of reaction which monitored by taking TLC the reaction mixture was added into ice-cold water. The solid separates out which was filtered and dried and re-crystallized from ethanol.

General procedure for the synthesis of 7-(**2-((2-substitutedbenzylidenehydrazinyl)oxy)-2-oxoethoxy)-4-methyl-2*H*-chromen-2-one (4)**

In 20ml dry DMF to added **7**-(2-hydrazinyloxy)-2-oxoethoxy)-4-methyl-2*H*-chromen-2-one (0.01mol). To this solution add equimolar amount of substituted aromatic aldehydes and 2-3 drop of glacial acetic acid. The resultant reaction mixture was refluxed at 100^{o}C for 22-24hrs, after completion of reaction which monitored by taking TLC, the reaction mixture was added into ice-cold water. The solid separates out which was filtered and dried, re-crystallized from ethanol.

General procedure for the synthesis of 4-methyl-7-((5-substituted phenyl-1,3,4-oxadiazol-2-yl)methoxy)-2*H*-chromen-2-one 5(a-h)

To a solution of 7-(2-((2-substitutedbenzylidenehydrazinyl)oxy)-2-oxoethoxy)-4-methyl-2*H*-chromen-2-one (4) in 10 ml of DMF and a catalytic amount of acetic acid. The resultant reaction mixture of was refluxed at 100^{o}C for 22-24hrs after completion of reaction which monitored by taking TLC, the reaction mixture was added into ice-cold water. The solid separates out which was filtered and dried and re-crystallized from ethanol and purified by colum chromatography (2:8 Ethyl acetate and petroleum ether).

4-methyl-7-((5-phenyl-1,3,4-oxadiazol-2-yl)methoxy)-2*H*-chromen-2-one (5a)

Yield: 65%, m. p. 272–274 ^{0}C; IR (KBr,cm^{-1}): 1632 (C=N), 1407 (N–N), 1247 (C–O–C). ^{1}HNMR (400MHz, DMSO- d_6): 2.42 (s,3H), 5.29 (s,2H), 6.23 (s,1H), 6.93 (d,1H,Ar-H), 6.97 (s,1H,Ar-H), 7.74 (d,1H, Ar-H), 7.48-8.10 (m,5H,Ar-H), MS (*m/z)* 335(M+1)$^+$.Anal.Calcd for $C_{19}H_{14}N_2O_4$; C,68.26;H,4.22; N,8.38; Found: C,68.16; H,4.21;N,8.35%.

7-((5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one(5b)

Yield: 45%, m. p. 253–255 ^{0}C; IR (KBr,cm^{-1}): 1649 (C=N), 1423 (N–N), 1261 (C–O–C). ^{1}HNMR (400MHz, DMSO- d_6):2.32(s,3H), 5.36 (s,2H), 5.49 (bs,1H), 6.32 (s,1H), 6.97(d,1H,Ar-H) 7.20(s,1H,Ar-H), 7.27-7.35(m,4H,Ar-H). 7.73 (d,1H,Ar-H), MS (*m/z)* 352 (M+1)$^+$. Anal. Calcd for $C_{19}H_{14}N_2O_5$; C,65.14; H,4.03; N,8.00; Found: C,65.10; H,3.98; N,7.69%.

7-((5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)methoxy)-4methyl-2*H*-chromen-2-one (5c)

Yield: 52%, m.p. 281-283 ^{0}C; IR (KBr,cm^{-1}):1643 (C=N), 1417 (N–N), 1257(C–O–C).^{1}HNMR (400MHz, DMSO- d_6):2.38(s,3H), 5.29 (bs,1H), 5.39(s,2H), 6.23 (s,1H), 7.02(d, 1H,Ar-H), 7.16(s,1H,Ar-H), 7.32 (d,2H,Ar-H), 7.45 (d, 2H,Ar-H),7.54(d,1H,Ar-H). MS (*m/z)* 351 (M+1)$^+$.Anal. Calcd for $C_{19}H_{14}N_2O_5$; C,65.14; H,4.03; N,8.00; Found:C,65.01; H,3.78; N,7.79%.

7-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2yl)methoxy)-4-methyl-2*H*-chromen-2-one (5d)

Yield: 55%, m. p. 248–250 ^{0}C; IR (KBr,cm^{-1}): 1615 (C=N), 1415 (N–N), 1242 (C–O–C). ^{1}HNMR (400MHz, DMSO- d_6):2.43(s,3H), 3.85 (s,3H), 5.42(s,2H), 6.19(s,1H), 6.90 (d,1H, Ar-H), 6.97 (s, 1H,Ar-H), 7.69(d,1H,Ar-H), 7.89 (d,1H,Ar-H), 7.92(d,1H,Ar-H), 8.02 (d,1H,Ar-H), 8.10(d,1H,Ar-H). MS (*m/z)* 365 (M+1)$^+$. Anal.Calcd for $C_{20}H_{16}N_2O_5$; C,65.93; H,4.43; N,7.69; Found:C,65.90;H,4.42;N,7.65%.

7-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one (5e)

Yield: 42%, m. p. 292–294 ^{0}C; IR (KBr,cm^{-1}): 1669 (C=N), 1447 (N–N), 1257 (C–O–C). ^{1}HNMR (400MHz, DMSO- d_6):2.32(s,3H), 5.35 (s,2H), 6.10 (s,1H), 6.90 (d,1H, Ar-H), 6.75(s, 1H,Ar-H), 7.70 (d,1H,Ar-H),7.84 (d,2H,Ar-H), 7.65(d,2H,Ar-H). MS (*m/z)* 368 (M+1)$^+$, 370 (M+2)$^+$.Anal. Calcd for $C_{19}H_{13}ClN_2O_4$;C,61.88; H,3.55; N,7.60; Found:C,61.85; H,3.51; N,7.55%.

4-methyl-7-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-2*H*-chromen-2-one (5f)

Yield: 55%, m.p. 266–268 ^{0}C; IR (KBr,cm^{-1}): 1669 (C=N), 1451 (N–N), 1278 (C–O–C). ^{1}HNMR (400MHz, DMSO- d_6):2.32(s,3H), 5.43 (s,2H), 6.18 (s,1H), 6.97 (d,1H, Ar-H), 7.10(s, 1H,Ar-H), 7.70(d,1H,Ar-H), 8.27(d,1H,Ar-H),8.32 (d,1H,Ar-H),8.38(d,1H,Ar-H), 8.45(d,1H,Ar-H). MS (*m/z)* 380 $(M+1)^{+}$. Anal. Calcd for $C_{19}H_{13}N_3O_6$; C,60.16; H,3.45; N,11.08; Found:C,60.08;H,3.45;N,11.00%.

4-methyl-7-((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-2*H*-chromen-2-one (5g)

Yield: 39%, m. p. 257–259 ^{0}C; IR (KBr,cm^{-1}): 1656 (C=N), 1448 (N–N), 1248 (C–O–C). ^{1}HNMR (400MHz, DMSO- d_6): 2.45 (s,3H), 5.20 (s,2H), 6.10 (s,1H), 7.10 (d,1H, Ar-H), 7.30 (s,1H,Ar-H), 7.70 (d,1H,Ar-H), 7.92-8.10 (m,4H,Ar-H). MS (*m/z)* 380 $(M+1)^{+}$.Anal. Calcd for $C_{19}H_{13}N_3O_6$; C,60.16; H,3.45; N,11.08; Found :C,60.02; H,3.35; N,10.95%.

4-methyl-7-((5-(m-tolyl)-1,3,4-oxadiazol-2-yl)methoxy)-2*H*-chromen-2-one (5h)

Yield: 61%, m. p. 280–282 ^{0}C; IR (KBr,cm^{-1}): 1615 (C=N), 1423 (N–N), 1215 (C–O–C). ^{1}HNMR (400MHz, DMSO-d_6): 2.32 (s,3H), 2.28 (s,3H), 5.14 (s,2H), 6.20 (s,1H), 7.20 (d,1H, Ar-H), 7.25 (d,1H, Ar-H), 7.40(t,1H, Ar-H), 7.65 (d,1H,Ar-H), 6.92 (s,1H,Ar-H), 7.79 (s,1H,Ar-H),7.86 (d,1H, Ar-H). MS (*m/z)* 349 $(M+1)^{+}$. Anal. Calcd for $C_{20}H_{16}N_2O_4$; C,68.96; H,4.63; N,8.04; Found :C,68.90; H,4.55; N,8.00%.

Antimicrobial activity

Compounds **5(a-h)** were initially screened for *in vitro* antibacterial activity against Gram positive bacterial strains (*Staphylococcus aureus*) and Gram negative bacteria strain (*E-Coli*) utilizing the agar diffusion assay20. The anti biotic drug, Ciprofloxacin was also used as positive control. Antibacterial activity screening for analogs and positive control was performed at a fixed concentration of 10µg/mL. All compounds exhibited antibacterial activity against Gram +Ve and Gram –Ve bacterial strains with Zones of inhibition (ZOI) ranging from 20 mm to 25 mm. Compound **5e** was identified as a potent antibacterial agent against all Gram +Ve and Gram –Ve bacterial strains. Compounds **5f** and **5g** also showed good antibacterial activity against all Gram +Ve and Gram –Ve bacterial strains compared to standard anti biotic drug, Ciprofloxacin (Table-1).

Analogs **5(a-h)** were also examined for antifungal activity against fungal strains i.e., *Aspergillus niger* and *Candida albicans.* The antifungal drug, Flucanazole was used as a positive control. The fungal strains were grown and maintained on sabouraud glucose agar

plates. The plates were incubated at 27 ^{0}C for 72 h and resulting zone of inhibitions (ZOIs) were measured. Antifungal screening for analogs and positive control was performed at a fixed concentration of 10µg/mL. Compounds **5e** identified the most potent antifungal agent against all fungal strains. The remaining compounds **5f** and **5g** showed good antifungal activity compared to standard antifungal drug, Flucanazole.

Table 1: Zone of inhibition of data for 5(a-h) against different bacteria and fungi at 10µg/mL concentration.

S.No	Compd.	Concentration In µg/mL	Antibacterial Antifungal Zone of inhibition in mm			
			S. aures	*E. Coli*	*C.albicans*	*A. niger*
1.	5a	10	12	15	10	09
2.	5b	10	13	11	14	08
3.	5c	10	08	05	11	13
4.	5d	10	12	07	06	11
5.	5e	10	22	18	23	21
6.	5f	10	20	19	21	20
7.	5g	10	18	19	23	21
8.	5h	10	10	09	07	11
9.	Ciprofloxacin	10	25	20	-	-
10.	Flucanazole	10	-	-	26	24

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REFERENCES

1. Kh .M. Daoud, Sh. S. I smaeel, J. of pure science, tikrit, 2007; 12: 167-170.
2. K. M. Khan et al. “Letter inorganic Chemistry", 2004; 1: 50-52.
3. Shawali, A.S, Abdallah, M.A, Zayed, M.E.M. Eur. J. Med. Chem., 2009; 44: 2106-2112.
4. Lokanatha Rai, K. M, Linganna, N. Farmaco, 2000; 55: 389-392.
5. Akhter, M, Husain, A, Azad, B, Ajmal, M. Eur. J. Med. Chem., 2009; 44: 2372-2378.
6. Sheriff, A. F, Hayam M. A, Ashour R. H. A, Razik, A. El, Fattah H. A. El, Fattah, A. El N, Nagwa, El-D. Bioorg. Med. Chem., 2009; 17: 2410-2422.
7. Tan, T. M. C, Chen, Y, Kong, K. H, Bai, J, Li, Y, Lim, G. S, Ang, T. H, Lam, Y, Antiviral Research, 2006; 71: 7-14.
8. Warrener, R. N. Eur. J. Org. Chem, 2000; 20: 3363-3380.

9. Rajasekaran, S, Rao, G.K, Pai, S. P. N, Ranjan, A, International Journal of Chem. Tech. Research, 2011; 3(2): 555-559.
10. Chemistry of coumarins http://pubs.org/doi/abs/10.1021/cr60113a001.2009.
11. Joule JA, Mills K, Smith GF, Heterocyclic chemistry, 3rd ed. Stanley thrones publishers Ltd., 1999; 177-8.
12. Venugopala KN, Jayashree BS, Indian Journal of Hetero cyclic Chemistry, 2003; 12: 307-10.
13. Irena Kostova, Curr. Med. Chem. Anti-Cancer Agents, 2005; 5: 29-46.
14. Sharma, R, Arya, V, J. Chem. Pharm. Res., 2011; 3(2): 204-212.
15. Murrey, R. D. H, Medez, D, Brown, S.A, The natural coumarins occurrences, chemistry and biochemistry*",* John Wiley Interscience, New York.
16. Pechmann H, Duisberg C. Novel synthesis of coumarins. Chem. Ber., 1884; 17: 929-936.