International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 784-788
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Synthesis and Anti-Microbial Activity of Novel 2-((4-Methyl-2-Oxo-2*H*-Chromen-7-Yl) Oxy)-*N*-(5-Substituted Phenyl-1*H*-Tetrazol-1-Yl) Acetamide Derivatives

S. Srinivas, B. Srinivas, P. Kavitha, S. Kavitha and M. Ravinder*
***Research Center, Department of Chemistry, Chaitanya Post Graduate College (Autonomous) Kishanpura, Hanamkonda, Warangal (T.S) India - 506 001.**

Received: 15 Mar 2019 / Accepted: 17 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** bandarisrinivas2005@gmail.com

Abstract
An efficient general method has been described for the synthesis of novel 2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy)-*N*-(5-substitutedphenyl-1*H*-tetrazol-1-yl) acetamide derivatives **5(a-g).** Compound 7-hydroxy-4-methyl coumarin **(1)** 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 **(4)** than followed by cyclization with sodium azide in dimethylformamide (DMF) and a few drops of glacial acetic acid to yield the title compounds **5(a-g)**. These analogs were evaluated for their anti-microbial activity against *Staphylococcus aureus (*Gram positive bacteria) *Escherichia Coli* (Gram Negative bacteria) and *Aspergillus niger*, *Candida albicans* (fungi). The analogs **5f** identified as potent activity and **5c, 5d** showed moderate activity against anti-microbial 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, tetrazoles, antibacterial strains, antifungal strains.

*****

INTRODUCTION
Tetrazole is a heterocyclic compound containing a carbon atom and four nitrogen atoms in a five-membered ring. A well-known tetrazole is MTT, which is dimethyl thiazolyl diphenyl tetrazolium salt which was used in MTT assay to quantify the respiratory activity of live cells in cell culture, although it kills cells in the process[1,2]. When tetrazole compounds became widely used in agriculture, bio chemistry, medicine, pharmacology, explosives and

other aspects, research began to develop rapidly[3,4]. The tetrazolyl functional group which was often considered as a carboxylic acid replacement in drugs, not only because the pKa is close, but it also has approximately the same planar delocalized system space requirements and it provided a maximum nitrogen content of any heterocyclic compound[5],their potential applications as anti-hypertensive, anti-allergic, anti-biotic and anti-convulsant agents[6–9]. Tetrazoles are also used as plant growth regulators, herbicides, and fungicides[10]. Tetrazole derivatives have potential for drug development for HIV or other immune diseases[11,12]. Additionally they have also application in photography[13] and specialty explosives[14]. They are resistant to metabolic degradation as well as to chemical oxidants[15]. The incorporation tetrazole 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.

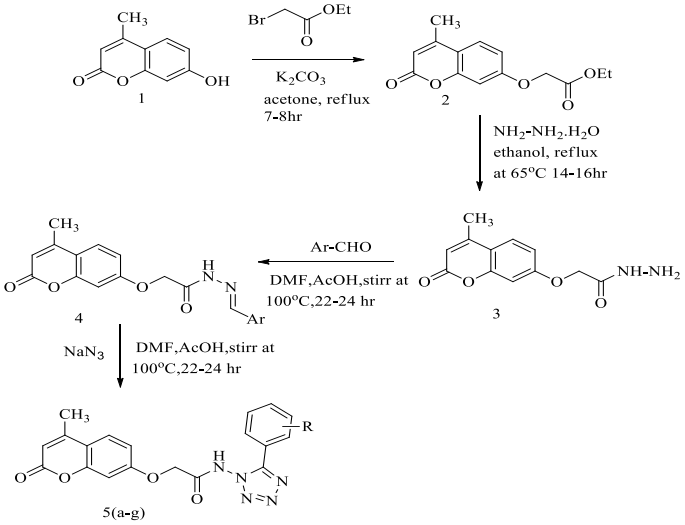
MATERIALS AND METHODS

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}HNMR 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 ethyl acetoacetate by Pechman condensation[16] method, which reacts with α-bromo ethyl acetate 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) acetohydrazide **(3).** The compound **3** is condensed with substituted aromatic aldehydes than followed by cyclization than followed by cyclization with sodium azide in dimethylformamide(DMF) and a few drops of glacial acetic acid to yield the title compounds **5(a-g)**. 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 **5(a)** showed characteristic absorption bands 1632 (C=N), 1407 (N–N), 3385 (-NH) absorption bands. The ^{1}H NMR spectrum of compounds **5(a)** showed peaks at δ 7.48 to 8.32 ppm due to aromatic protons and exhibited two singlet signals at δ 2.43 ppm due to one methyl protons another singlet at δ 4.29 ppm for –CH_2 protons. The –NH proton exhibit a broad singlet at δ 10.25 ppm.

Scheme:



S.No	Compound	Ar-R
1.	5a	Ar
2.	5b	2-OCH_3
3.	5c	4-OCH_3
4.	5d	2-OH
5.	5e	4-OH
6.	5f	4-Cl
7.	5g	4-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, then 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°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°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 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)-*N*-(5-ubstitutedphenyl-1*H*-tetrazol-1-yl) acetamide 5(a-g):

A mixture of 7-(2-((2-substitutedbenzylidenehydrazinyl) oxy)-2-oxoethoxy)-4-methyl-2*H*-chromen-2-one (0.01mol) and sodium azide (0.02 mol) in dimethyl form amide (10ml), catalytic volume of acetic acid were refluxed at 80°C to 100 °C for 22-24 hr. the progress of the reaction was monitored by TLC, after completion of the reaction, it was cooled to room temperature, poured into crushed ice, solid separated filtered, washed with water and re-crystallized with chloroform, purified by colum chromatography (3:7 Ethyl acetate and petroleum ether).

2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy)-*N*-(5-phenyl-1*H*-tetrazol-1-yl) acetamide (5a):

Yield: 59%, m. p. 292–294 °C; IR (KBr, cm^{-1}): 1632 (C=N), 1407 (N–N), 3385 (-NH)**.** ^{1}HNMR (400MHz, DMSO- d_6): 2.43 (s, 3H, -CH_3), 4.29 (s, 2H), 6.25 (s, 1H), 6.93 (d, 1H, Ar-H), 6.97 (s, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 7.48-8.32 (m, 5H, Ar-H), 10.25 (bs, 1H, -NH). MS (*m/z)* 378(M+1) $^+$. Anal.Calcd for $C_{19}H_{15}N_5O_4$; C,60.47; H,4.01; N,18.56; Found: C,60.43; H,3.96; N,18.53%.

***N*-(5-(2-methoxyphenyl)-1*H*-tetrazol-1-yl)-2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy) acetamide (5b):**

Yield: 54%, m.p. 275–277°C; IR (KBr, cm^{-1}): 1612 (C=N), 1427 (N–N), 3342 (-NH)**.**^{1}HNMR (400MHz, DMSO- d_6): 2.32 (s, 3H, -CH_3),3.92 (s, 3H-OCH_3), 4.72 (s, 2H), 6.45 (s, 1H), 6.92 (d, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 7.78 (d, 1H, Ar-H), 7.12-7.83 (m, 4H, Ar-H), 10.46 (bs, 1H, -NH). MS (*m/z)* 408 (M+1)$^+$. Anal.Calcd for $C_{20}H_{17}N_5O_5$; C,58.97; H,4.21; N,17.19; Found: C,58.93; H,4.19; N,17.19%.

***N*-(5-(4-methoxyphenyl)-1*H*-tetrazol-1-yl)-2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy) acetamide (5c):**

Yield: 44%, m.p.265–267°C; IR (KBr, cm^{-1}): 1619 (C=N), 1428 (N–N), 3349 (-NH). ^{1}HNMR (400MHz, DMSO- d_6): 2.45 (s, 3H, -CH_3),4.05 (s, 3H, -OCH_3), 4.63 (s, 2H), 6.37 (s, 1H), 6.89 (d, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 7.73 (d, 1H, Ar-H), 7.10-7.95 (d, 2H, Ar-H), 7.05-7.68 (d, 2H, Ar-H), 10.38 (bs, 1H, -NH). MS (*m/z)* 408 (M+1) $^+$. Anal.Calcd for $C_{20}H_{17}N_5O_5$; C,58.97; H,4.21; N,17.19; Found: C,58.95; H,4.20; N,17.16%.

***N*-(5-(2-hydroxyphenyl)-1*H*-tetrazol-1-yl)-2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy) acetamide (5d):**

Yield: 51%, m.p.288–290°C; IR (KBr, cm^{-1}): 1656 (C=N), 1438 (N–N), 3423 (-NH). ^{1}HNMR (400MHz,

DMSO- d_6): 2.48 (s, 3H, -CH_3),4.72 (s, 2H), 5.98 (bs, 1H, -OH), 6.38 (s, 1H), 6.97 (d, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 7.12-7.65 (m, 4H, Ar-H),10.21(bs, 1H, -NH). MS (*m/z)* 394 $(M+1)^+$. Anal.Calcd for $C_{19}H_{15}N_5O_5$; C,58.01; H,3.84; N,17.80; Found: C,57.95; H,3.80; N,17.76%.

_N_-(5-(4-hydroxyphenyl)-1_H_-tetrazol-1-yl)-2-((4-methyl-2-oxo-2_H_-chromen-7-yl) oxy) acetamide (5e):

Yield: 53%, m.p.272–274^{0}C; IR (KBr, cm^{-1}): 1649 (C=N), 1426 (N–N), 3453 (-NH).^{1}HNMR (400MHz, DMSO- d_6): 2.45 (s, 3H, -CH_3),4.82 (s, 2H), 6.10 (bs, 1H, -OH), 6.42 (s, 1H), 7.05 (d, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.89 (d, 1H, Ar-H), 6.66-7.82 (d, 2H, Ar-H),6.87-7.90 (d, 2H, Ar-H), 10.45(bs, 1H, -NH) MS (*m/z)* 394 $(M+1)^+$. Anal.Calcd for $C_{19}H_{15}N_5O_5$; C,58.01; H,3.84; N,17.80; Found: C,57.97; H,3.83; N,17.69%.

_N_-(5-(4-chlorophenyl)-1_H_-tetrazol-1-yl)-2-((4-methyl-2-oxo-2_H_-chromen-7-yl) oxy) acetamide 5(f):

Yield: 47%, m.p.284–286^{0}C; IR (KBr, cm^{-1}): 1655 (C=N), 1448 (N–N), 3485 (-NH). ^{1}HNMR (400MHz, DMSO- d_6): 2.52 (s, 3H, -CH_3),4.78 (s,2H), 6.32 (s,1H), 7.12 (d, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 7.66-8.32 (d, 2H, Ar-H),6.74-8.41 (d, 2H, Ar-H), 10.27 (bs, 1H, -NH). MS (*m/z)* 412 $(M+1)^+$, 414 (M+1). Anal.Calcd for $C_{19}H_{14}ClN_5O_4$; C,55.42; H,3.43; N,17.01; Found: C,55.40; H,3.41; N,16.69%.

2-((4-methyl-2-oxo-2_H_-chromen-7-yl) oxy)-N-(5-(p-tolyl)-1_H_-tetrazol-1-yl) acetamide 5 (g):

Yield: 62%, m.p.252–254^{0}C; IR (KBr, cm^{-1}): 1632 (C=N), 1414 (N–N), 3365 (-NH). ^{1}HNMR (400MHz, DMSO- d_6): 2.45 (s, 3H, -CH_3), 2.45 (s, 3H, -CH_3),4.68 (s, 2H), 6.22 (s, 1H), 6.72 (d, 1H, Ar-H), 7.12 (s, 1H, Ar-H), 7.83 (d, 1H, Ar-H), 7.32-8.44 (d, 2H, Ar-H),7.44-8.48 (d, 2H, Ar-H), 10.02 (bs,1H, -NH). MS (*m/z)* 392 $(M+1)^+$. Anal.Calcd for $C_{20}H_{17}N_5O_4$; C,61.38; H,4.38; N,17.89; Found: C,61.36; H,4.37; N,17.87%.

Antimicrobial activity

Compounds **5(a-g)** 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, Ampicillin was also used as positive control. Antibacterial activity screening for analogs and positive control was performed at a fixed concentration of 10µg/mL and 20µg/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 **5f** was identified as a potent antibacterial agent against all Gram +Ve and Gram –Ve bacterial strains. Compounds **5c** and **5d** also showed good antibacterial activity against all Gram +Ve and Gram –Ve bacterial strains compared to standard anti-biotic drug, Ampicillin **(Table-1).**

Compounds **5(a-g)** were also examined for antifungal activity against fungal strains i.e., *Aspergillus niger* and *Candida albicans.* The antifungal drug, Ketaconazole 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 and 20µg/mL. Compounds **5f** identified the most potent antifungal agent against all fungal strains. The remaining compounds **5c** and **5d** showed good antifungal activity compared to standard antifungal drug, Ketaconazole.

Table-1: Zone of inhibition of data for 5(a-g) against different bacteria and fungi at 10µg/mL and 20µg/mL concentration.

S.No	Compds.	Concent. µg/ml	Anti-bacterial Anti-fungal Zone of inhibition in mm			
			S.*aures*	E.*Coli*	C.*albicans*	A. *niger*
1.	5a	10	01	01	-	-
		20	02	01	-	-
2.	5b	10	06	04	-	-
		20	04	08	-	-
3.	5c	10	12	14	07	10
		20	15	13	12	11
4.	5d	10	13	12	12	11
		20	17	14	11	10
5.	5e	10	07	05	02	03
		20	12	10	04	05
		10	20	18	17	16

6.	5f	20	17	18	16	15
		10	02	05	04	04
7.	5g	20	05	02	06	04
8.	Ampicillin	5	22	18	-	-
9.	Ketaconazole	5	-	-	19	18

CONCLUSION:

We have synthesized novel 2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy)-*N*-(5-substitutedphenyl-1*H*-tetrazol-1-yl) acetamide derivatives **5(a-g)**. All the synthesized compounds **5(a-g)** were screened for anti-microbial activity. Among all the synthesized compounds **5f** showed good activity and **5c,5d** exhibited moderate activity against gram-positive bacterial strains *S. aureus* and gram-negative bacterial strains *E.coli*. **5f** showed good activity and compounds **5c, 5d** and showed moderate activity against *C. Albicans* and *A. niger*.

ACKNOWLEDGEMENTS

The authors are thankful to the Minor Research Project (UGC-SERO), Hyderabad for providing financial support and also thankful to our Chairman, Dr. Ch. V. Purushottam Reddy, Principal, Dr. K. Veeravenkatiah for providing research facilities.

REFERENCES:

1. B. Dahlof, R.B., Deveruex, S.E., Kjeldsen. Cardiovascular morbidity and mortality in the Losartan Intervention for endpoint reduction in hypertension study, a randomized trial against atenolol. Lancet. 2002, 359, 9311, 995-999.
2. T. Mosmann., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth. 1983, 65, 55-63.
3. Su, W.K., Hong, Z., Shan, W.G., Zhang, X.X., A facile synthesis of 1-substituted-1*H*-1,2,3,4-tetrazoles catalyzed by ytterbium triflate hydrate. Eur. J. Org. Chem. 2006, 37, 2723–2726.
4. Benson, F.R., Theoretical principles of the chemistry of heterocycles. In Heterocyclic Compounds; John Wiley & Sons, Inc. New York, NY, USA, 1967; p. 1.
5. Demko, Z.P., Sharpless, K.B., Preparation of 5-substituted-1*H*-tetrazoles from nitriles in water. J. Org. Chem. 2001, 66, 7945–7950.
6. Berghmans, S., Hunt, J., Roach, A., Goldsmith, P. Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Res. 2007, 75, 18–28.
7. Toney, J.H., Fitzgerald, P.M., Grover-Sharma, N., Olson, S.H., May, W.J., Sundelof, J.G., Vanderwall, D.E., Cleary, K.A., Grant, S.K., Wu, J.K. Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of Bacteroides fragilis metallo-beta-lactamase. Chem. Biol.1998, 5, 185–196.
8. Butter, R.N., Katritzky, A.R., Rees, C.W., Eds. Pergamon Press: New York, NY, USA, 1984. 5, 4A, p. 791.
9. Tamura, Y., Watanabe, F., Nakatani, T., Yasui, K., Fuji, M., Komurasaki, T., Tsuzuki, H., Maekawa, R., Yoshioka, T., Kawada, K. Highly selective and orally active inhibitors of type IV collagenase (MMP-9 and MMP-2): *N*-sulfonylamino acid derivatives. J. Med. Chem.1998, 41, 640–649.
10. G. Sandmann, C., Schneider, P.Z. Boger., C. Naturforsch., A new non-radioactive assay of phytoene desaturase to evaluate bleaching herbicides. Bioscience. 1996, 51, 534.
11. E. Wood., R.M. Crosby., S. Dickerson., S.V. Frye., R. Griffin., R. Hunter. A prodrug approach to the design of cRaf1 kinase inhibitors with improved cellular activity. Anti-Cancer Drug Des. 2001, 16, 1.
12. B.C.H. May., A.D. Abell., α-methylene tetrazol-based peptidomimetics: synthesis and inhibition of HIV protease. J. Chem. Soc. Perkin. Trans. 2002, 2, 172.
13. R.J. Herr., 5-Substituted-1H-tetrazoles as carboxylic acid isosteres: medicinal chemistry and synthetic methods. Bioorg. Med. Chem. 2002, 10, 3379.
14. R.P. Singh., R.D. Verma., D.T. Meshri., J.M. Shreeve., Energetic nitrogen-rich salts and ionic liquids Angew. Chem. Int. Ed, 2006, 45, 3584.
15. T. Eicher, S., Hauptmann. The Chemistry of Heterocycles. 1995**.** 212.
16. Pechmann H, Duisberg C. Novel synthesis of coumarins. Chem. Ber. 1884, 17,929-936.