Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (6): 2592-2597

(International Peer Reviewed Journal)

Water-Mediated Green Economical Synthesis of Biscoumarins And Their Cell Cytotoxic Activity

Thechano Merry[1], Palakolanu. S. Reddy[2], Anand K. Kondapi[2] and Maddela Prabhakar*[1]

1. Department of Chemistry, Nagaland University, Lumami-798627, Nagaland, **INDIA**
2. Department of Biotechnology& Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad-500046, **INDIA**

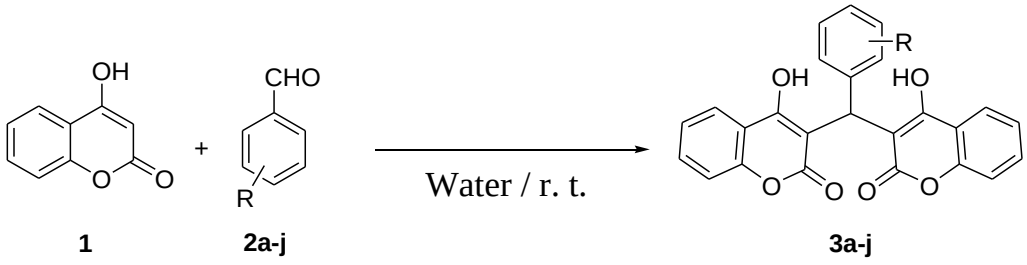
Email: drprabhakarm@gmail.com

Accepted on 15th November 2014

ABSTRACT

Water-mediated efficient and economical green protocol for the synthesis of Biscoumarin derivatives in excellent yields at room temperature and their cell cytotoxic activity investigated. These water-mediated reactions of 4-hydroxycoumarin with substituted aldehydes avoids the use of any catalysts, toxic solvents, heating and provides several advantages such as simple, economical, eco-friendly and practically scalable green synthesis.

Graphical Abstract



Keywords: Water-mediated, Catalyst-free, Green Chemistry, Biscoumarins, 4-Hydroxycoumarin, Aldehydes, Cell Cytotoxic Activity.

INTRODUCTION

Coumarins owe their class name to 'Coumarou', the name comes from a French term for the Tonka bean (*Dipteryx odorata* Willd., Fabaceae), one of the sources from which the substance was first isolated as a natural product in 1820[1]. Coumarins, a class of compounds that contains a 1, 2-benzopyrone skeleton, are widespread in plants including many vegetables, spices, fruits, and medicinal plants[2]. The biosynthesis of coumarin in plants is via hydroxylation, glycolysis and cyclization of cinnamic acid. More than 1300 coumarins were identified from natural sources[3]. Most of these compounds are not harmful to humans in the amounts present in edible plants [4].

The synthesis of coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus[5]. Coumarin derivatives have been reported for anticoagulant, anti-inflammatory, antimicrobial[6], antioxidant[7], anti-allergic, anticancer[8] and anti-proliferative and antiviral[9] activities. They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals[10] and optical brighteners[11] and dispersed fluorescent and laser dyes[12]. Thus the synthesis of this heterocyclic nucleus is of much interest. Coumarins have been synthesized by several routes including Pechmann[13], Perkin[14], Knoevenagel[15], Reformatsky[16] and Wittig[17] reactions.

Currently, the synthetic chemists are more challenged to consider more environmentally friendly methods for generation of the desired target molecules. In green chemistry, the desire for to utilize ‘‘safer solvents’’ and to ‘‘design for energy efficiency’’ can be considered 2 key principles of relevance to synthetic chemists[18]. Because of the toxic and volatile nature of many organic solvents, water as a reaction medium was considered a very promising and attractive substitute for volatile organic solvents and was widely used in the green chemistry area since Breslow[19], who showed that hydrophobic effects could strongly enhance the rate of several organic reactions, rediscovered the use of water as a solvent in organic chemistry in 1980s.

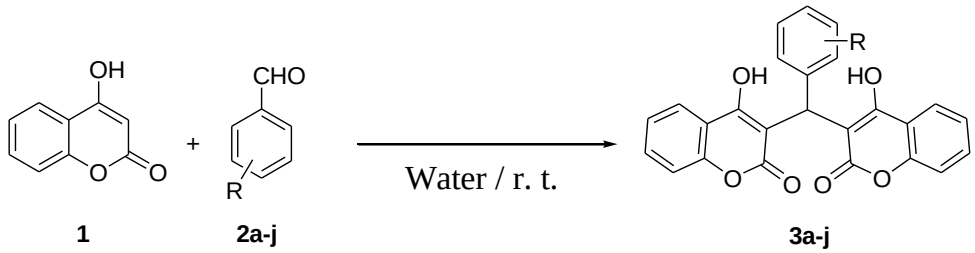
Biscoumarins are naturally occurring compounds[20-22] and also synthesized by different methods[23-25]. Recently, few numbers of methods has been reported for the synthesis of bis(4-hydroxycoumarin-3-yl) toluene using 4-hydroxycoumarin with various aldehydes in aqueous media. Despite effectiveness and eco-friendliness of these methods, they use catalysts such as TEBA[26] and have long reaction times.

Thus, the search and introduction of new efficient green methodology is still in great demand in industry as well as in academia. In this context, continuation of our on-going research for the development of simple and efficient methods for the synthesis of organic compounds, herein we report an economically efficient water-mediated green method for the synthesis of biscoumarin derivatives at room temperature.

MATERIALS AND METHODS

General: All reagents and solvents were of the highest commercial quality purchased from Aldrich & Merk and were used without further purification. Thin layer chromatography (TLC) was performed on silica gel glass plates. Chromatograms were visualized by, UV at 254 and 365 nm, followed by iodine vapors.Melting points were determined on a *Kofler* hot-stage apparatus and are uncorrected.

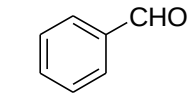
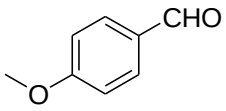
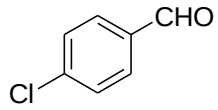
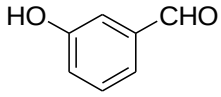
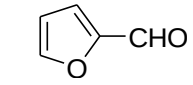
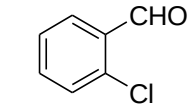
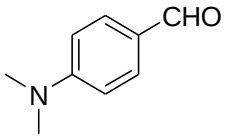
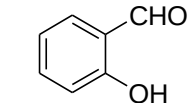
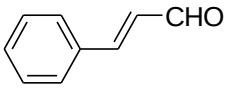
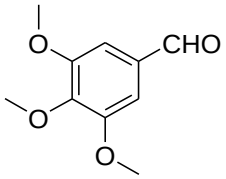
Synthesis: General procedure for the synthesis of Biscoumarin derivatives (**3a-j**): To a mixture of 4-hydroxycoumarin (**1**) (2 mmol) and aromatic/heteroaromatic aldehydes (**2a-j**) (1 mmol) was added 15 mL of water and stirred at room temperature for the appropriate time mentioned in table 1. The completion of reaction was monitored by Thin Layer Chromatography system. After completion of the reaction, the solid products were collected by filtration methods, finally washed with water and recrystallized from ethanol-water to give the desired pure products (**3a-j**) (Scheme 1). All synthesized compounds TLC recorded were compared with the corresponding literature TLC and found to be matching.



1 **2a-j** **3a-j**

Scheme 1. Water-mediated synthesis of Biscoumarin derivatives at room temperature

Table 1. Synthesis of Biscoumarins **3a-j** in Water.[a]

Entry	Aldehyde	Product	Time/h	Melting Point/^{o}C (Literature m.p./^{o}C)	Yield (%)
1		**3a**	1h	226–229(228–230)	98
2		**3b**	4h	243–245 (242–244)	96
3		**3c**	3h	258–260 (259–261)	95
4		**3d**	6h	208–211 (210–211)	94
5		**3e**	3h	200–203 (199–201)	94
6		**3f**	4h	240–241 (239–240)	96
7		**3g**	6h	207–209 (208–210)	94
8		**3h**	6h	255–257 (254–256)	93
9		**3i**	6h	230–233 (230–232)	94
10		**3j**	4h	156–158 (157-159)	96

[a]Reaction conditions: 4-hydroxycoumarin (2.0 mmol); Aldehyde (1.0 mmol); Water (15 mL) at room temperature.

RESULTS AND DISCUSSION

As a part of our research in developing new versatile and efficient green methods for the synthesis of organic compounds, herein we report efficient eco-friendly economical green synthetic method for the synthesis of biscoumarin derivatives starting from 4-hydroxycoumarin with substituted aldehydes in water-

media at room temperature (Scheme 1) and their cell cytotoxicity studies (Table 3). In our initial study, we performed the reported reaction of biscoumarin starting from4-hydroxycoumarin with benzaldehyde in the presence of iodine in water under refluxing temperature for 30 min[27]. The expected product was obtained in 95% yield and the melting point was recorded to confirm the product which was matching to the reported one. In order to standardize our method we also accomplished the reported reactions of anisaldehyde and 4-chlorobenzaldehyde under solvent-free thermal conditions [28] and confirm the products by comparing the TLC and melting points. Further we also investigated the use of iodine and other catalysts like acetic acid, ammonium acetate and citric acid for the synthesis of biscoumarin to identify the role of the catalysts in the water reaction media at room temperature (Table 2, entries 1-4) and these results suggesting that the catalysts are slowing the reaction process and yields. After our several systematic investigations, we astonishingly found the formation of desired product in water media without any catalyst. We carefully observed and optimized the reaction condition, and the excellent yield was achieved when the reaction was carried out in absence of catalyst at room temperature in water-media and the reaction was completed within 1h yielded 98% (Table 2, entry 5). To study the effects of reaction parameters such as protic solventsethanol, methanol and aprotic solvents such as acetonitrile and dichloromethane, none of these solvents were performed the reaction. These findings conclude that the water is acting as a catalyst for the synthesis of biscoumarins.

Table 2.Evaluation of catalysts for the synthesis of Biscoumarin **3a** in water.[a]

Entry	Catalyst	Mol (%)	Time	Yield (%)
1	I_2	10	24h	65
2	Acetic acid	10	24 h	40
3	NH_4OAc	10	24 h	60
4	Citric acid	10	24h	50
5	**Water**	**—**	**1h**	**98**

[a]Reaction conditions: 4-hydroxycoumarin (2.0 mmol); Benzaldehyde (1.0 mmol); Catalyst; Water (15 mL) at RT.

To rationalize the standardized economical green synthetic method, we further explore the experiments with various substituents on the aromatic ring (both electron donating and electron withdrawing) have not shown much effect on the formation of final products in terms of yields under these reaction conditions (Table 1, 10).

APPLICATIONS

Cell Cytotoxicity Assay of Biscoumarin Derivatives (3a-j): Cell cytotoxicity studies and percentage of cell cytotoxicity was given in Table 3. Method for cell cytotoxicity assay as follows: 0.2 Million cells (SupT-1) were seeded in each well in a 96-well cell culture plate (Orange Scientific). The plates were maintained at appropriate conditions in an incubator (Forma Scientific). The cells were pelleted by centrifugation at 1000 rpm for 10 min and supernatant was removed. 180 µL complete medium with 20 µl inhibitor (1ηM, 100ηM and 10µM) was added to the cell pellet and incubated for 16 h. Thereafter the plate was centrifuged and media along with the inhibitor was removed. 20 µL of MTT (5mg mL^{-1} stock) along with 180 µL complete media was added and incubated for 4-8 h. The plate was again centrifuged at 1000 rpm for 10 min and the supernatant was removed. 200 µL of DMSO/acidic isopropanol was added and left at room temperature for 5-10 min. The absorbance was recorded at 570 ηm using a plate reader.

Table 3. Cell Cytotoxicity Assay of Biscoumarin Derivatives **3a-j**.

S. No.	Biscoumarin	Solubility	Concentration	% of Cell Cytotoxicity
1	**3a**	DMSO	1nM	56.69
2	**3b**	DMSO	1nM	57.37
3	**3c**	DMSO	1nM	48.51
4	**3d**	DMSO	1nM	39.34
5	**3e**	DMSO	10µM	23.61
6	**3f**	DMSO	1nM	33.75
7	**3g**	DMSO	1nM	32.25
8	**3h**	DMSO	1nM	45.33
9	**3i**	DMSO	10µM	24.93
10	**3j**	DMSO	1nM	50.52

CONCLUSIONS

In conclusion, we have developed a simple water-mediated green protocol for the synthesis of biscoumarins at room temperature and the present methodology was superior to the literature methods in terms of catalyst-free, water-mediated efficient scalable green economical synthesis and investigated their biological studies towards cell cytotoxicity.

ACKNOWLEDGMENTS

TM and MP thank the Vice-Chancellor, Dean RDC, Dean School of Sciences, Head, Faculty and Staff of the Chemistry Department, Nagaland University. MP specially thanks the University of Hyderabad for providing scientific facilities for this work.

REFERENCES

[1] J. Bruneton, *Pharmacognosy, Phytochemistry, Medicinal Plants (2nd Ed)*, **1999**, 263-277.
[2] A. Vogel, *Annalen der Physik*, **1820**, 64(2), 161-66.
[3] R. D. H. Murray, *Prog. Chem. Org. Nat. Prod*, **2002**, 83, 1−673.
[4] J. R. Hoult, M. Paya, *Gen. Pharmacol*, **1996**, 27, 713-22.
[5] D. Egan, R. O'Kennedy, E. Moran, D. Cox, E. Prosser, R. D, Thornes, *Drug Metab. Rev*, **1990**, 22, 503−529.
[6] B. Rajitha, V. K. Naveen, P. Someshwar, J. M. Venu, P. R. Narsimha, Y. Reddy, *ARKIVOC.*, **2006**, (XII), 23-27.
[7] V. V. Mulwad, J. M. Shirodkhar, *Indian Journal of Heterocyclic Chemistry*, **2002**, 11, 192-202.
[8] K. Manohar, G. Manjunath, K. Raviraj, *Indian Journal of Heterocyclic Chemistry*, **2004**, 13, 201-204.
[9] V. R. Rao, K. Srimanth, P. V. Kumar, *Indian Journal of Heterocyclic Chemistry*, **2004**,14, 141-144.
[10] Z. M. Nofal, M. I. El-Zahar, S. S. Abd El-Karim, *Journal of Antimicrobial Chemotherapy*, **2005**, 5, 483–488.
[11] R. O'kennedy; R. D. Thornes, *Coumarins: Biology application and mode of action;* John Wiley and sons: Chichester, **1997**.

[12] M. Zabradnic, *The production and application of fluorescent brightening agents;* John wiley and sons: Newyork, **1992**.
[13] R. D. Murreey, J. Mendez, S. A. Brown, *The natural coumarins: occurrence, chemistry and biochemistry;*John wiley and sons: Newyork, **1982**.
[14] H. V. Pechmann, C. B. Duisberg, *Ber*, **1884**, 17, 929.
[15] J. R. Johnson, *Org. React*., **1942**, 1, 210.
[16] G. Jones, *Org. React*, **1967**, 15, 204.
[17] G. Brafola, F. Fringuelli, O. Piermatti, F. Pizzo, *Heterocycles*, **1996**, 43, 1257.
[18] D. Dallinger, C. O. Kappe, Microwave-assisted synthesis in water as solvent.*Chem. Rev*, **2007**, 107, 2563–2591.
[19] (a) R. Breslow, D. C. Rideout, Hydrophobic acceleration of Diels–Alder reactions, *J. Am. Chem. Soc*, **1980**, 102, 7816–7817. (b) R. Breslow, Hydrophobic effects on simple organic reactions in water.*Acc. Chem. Res*, **1991**, 24, 159–164.
[20] K. P. Link, *Fed. Proc*, **1945**, 4, 176.
[21] P. Sengupta, M. Sen, P. Karuri, *J Ind. Chem. Soc*, **1985**, 62, 916.
[22] R. D. H. Murray, *Fortschr Chem. Org. Naturst*, **1991**, 58, 83.
[23] R. A. Abramovitch, J. R. Gear, *Can. J.* Chem, **1958**, 36, 1501.
[24] L. Wolff, *Ann*., **1901**, 315, 145.
[25] R. Anschu¨ tz; J. Wagner, P. Junkersdorf, *Ann*, **1909**, 367, 217.
[26] J. Wang, D. Q. Shi, Q. Y. Zhuang, X. S. Wang, S. J. Tu, *J. Org. Chem*, **2005**, 25, 926–929.
[27] K. Mazaahir, V. Bansal, P. Mothsra, S. Saxena, R. K. Somvanshi, S. Dey, T. P. Singh, *J. Mol. Catal. A—Chem*., **2007**, 268, 76–81.
[28] S. Hamid Reza, H. Moones, *Chinese Journal of Chemistry*, **2009**, 27, 1795-1800.