Taylor & Francis
Taylor & Francis Group

Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: https://www.tandfonline.com/loi/lsyc20

An efficient one-pot synthesis of coumarin-amino acid derivatives as potential anti-inflammatory and antioxidant agents

Mamata Devendra Naik, Yadav D. Bodke, Vijay Kumar M & Revanasiddappa BC

To cite this article: Mamata Devendra Naik, Yadav D. Bodke, Vijay Kumar M & Revanasiddappa BC (2020): An efficient one-pot synthesis of coumarin-amino acid derivatives as potential anti-inflammatory and antioxidant agents, Synthetic Communications, DOI: 10.1080/00397911.2020.1735442

To link to this article: https://doi.org/10.1080/00397911.2020.1735442

SYNTHETIC COMMUNICATIONS®
https://doi.org/10.1080/00397911.2020.1735442

An efficient one-pot synthesis of coumarin-amino acid derivatives as potential anti-inflammatory and antioxidant agents

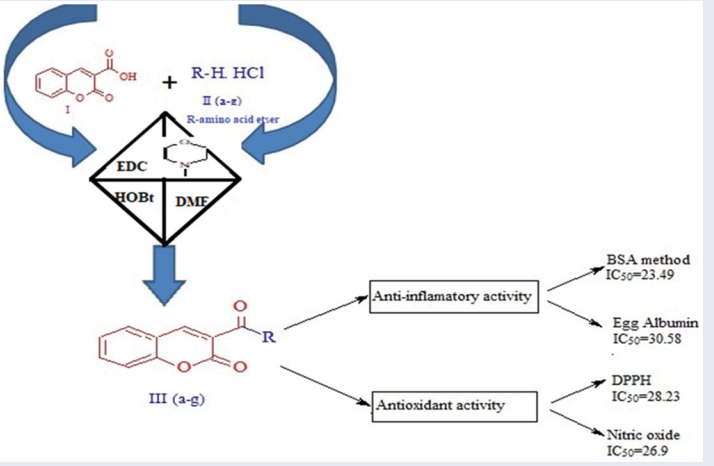
Mamata Devendra Naik[a], Yadav D. Bodke[b], Vijay Kumar M[c], and Revanasiddappa BC[c]

[a]Department of P.G. Studies and Research in Industrial Chemistry, Jnanasahyadri, Kuvempu University, Shimoga, India; [b]Department of P.G. Studies and Research in Chemistry, Jnanasahyadri, Kuvempu University, Shimoga, India; [c]Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, NITTE, Mangalore, India

ABSTRACT

A series of seven coumarinyl-amino acid ester conjugates have been synthesized and characterized by NMR (^{1}H and 13C) and mass spectra. Further, the compounds were investigated for their therapeutic applications such as anti-inflammatory and antioxidant activities. Among the synthesized compounds most of the analogs showed good efficiency compared with the standard.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 6 November 2019

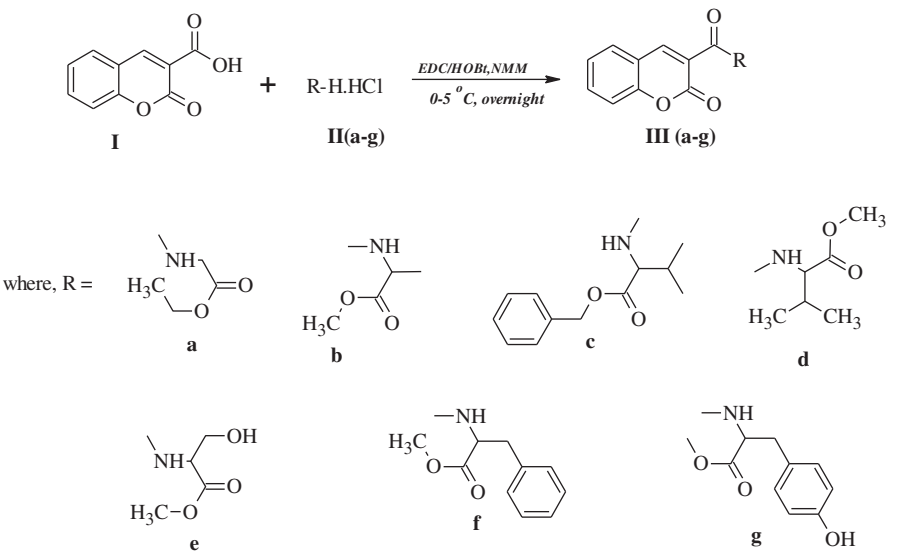
KEYWORDS
Amino acid ester; anti-inflammatory activity; antioxidant activity; conjugates; coumarin-3-carboxylic acid

Introduction

Coumarins are some versatile heterocyclic nuclei which constitute one of the important structural motifs of natural products. The analogs of coumarins are synthesized by a variety of methods to enhance its bio-medicinal properties. Coumarin is found to be

CONTACT Yadav D. Bodke ydbodke@gmail.com Department of P.G. Studies and Research in Chemistry, Jnanasahyadri, Kuvempu University, Shimoga, Karnataka 577451, India.

Supplemental data for this article can be accessed on the publisher's website



Scheme 1. The synthetic pathway involved in the synthesis of target peptides **III (a–g)**.

one of the key molecular templates which block the inflammation by inhibiting lipoxygenase (LOX), cyclooxygenase (COX) which are produced in the pathways of arachidonic acid metabolism and also by other mechanisms. Various other contributions made on coumarins also justify its role in the development of molecules possessing anti-inflammatory activity. Coumarin compounds from both plant and synthetic origin are found to possess the special capacity to scavenge reactive oxygen species (ROS) and regulate stress-related chronic diseases.[1–2] The substitution at the third and fourth position of coumarin nucleus have been studied more because of its great utility in the medicinal field and drug discovery.[3–5] Coumarin-3-carboxylic acid has been used as one of the key synthon for several natural/synthetic pharmacological agents.[6]

Acid amine coupling is a well-recognized tool in peptide synthesis. Amino acids are the essential constitutes of most of the proteins which are building blocks of human life. Conjugation of amino acids with other biologically active scaffolds is found to enhance their physiological effects.[7–9] The high bioavailability, low toxicity and effective biological activities of peptide derivatives have opened up new perspectives in drug design and the field of biomedical research.[10]

The presence of amide functionality in a large array of biological molecules signifies their role in modern biology.[11] Activation of the carboxylic group using different coupling reagents is found to be a more convenient method than converting carboxylic acid to acid chlorides followed by the reaction with amine for peptide synthesis. There are several coupling agents available in the market and one to select an appropriate coupling agent based on the mechanism through which it acts on substrates. There are many literature reports which justify that the presence of an amide bridge linker between coumarin and other heterocycles is associated with many biological activities.[12–14]

Keeping in view the above, the therapeutic applications of amino acids and coumarin scaffolds in the modern drug discovery, this work pertains simple and convenient acid

amine coupling reaction involving coumarin-3-carboxylic acid and different amino acid esters as depicted in Scheme 1. Further, the synthesized scaffolds were evaluated for *in vitro* antioxidant and anti-inflammatory activity.

Result and discussion

Chemistry

The desired bioactive conjugates were synthesized by the well-known acid amine coupling reaction. One of the key synthon required for the synthesis of target compounds, coumarin 3-carboxylic acid **I** was synthesized by reported procedure[13] and coupled with different commercially available amino acid esters of *L*-configuration (glycine ethyl ester hydrochloride, alanine methyl ester hydrochloride, valine benzyl ester hydrochloride, leucine methyl ester hydrochloride, serine methyl ester hydrochloride, phenylalanine methyl ester hydrochloride, and tyrosine methyl ester hydrochloride) to get desired conjugated molecules **III(a–g)** as shown in Scheme 1.

Amide functionality is obtained by the condensation of carboxylic acid and amine group. In this reaction, the elimination of water molecules is necessary. Therefore, these reactions were performed using certain coupling agents at ambient temperature. Thus in the present investigation, we have used EDC.HCl/HOBt as a coupling agent in the presence of N-methyl morpholine as a base. The carboxylic group is activated by treating it with carbodiimide to convert it to a good leaving group prior to coupling with the amino acid ester. HOBt helps in reducing the racemization by converting the O-acyl urea to activated ester. The ester so formed reacts with amines to give the corresponding amide.[15] Further, the synthesized compounds were well characterized by different spectral techniques and the spectral data of the synthesized compounds were in accordance with expected values (see Supplementary information).

The postulated structure of synthesized methyl-3-hydroxy-2-[(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] propanoate (**IIIe)** was confirmed by FT-IR, ^{1}H NMR, 13C NMR and mass spectral data. The FT-IR spectrum of compound (**IIIe)** showed an absorption band at 3321 cm^{-1} attributed to the –NH stretching of the AMIDE I linkage. The other stretching vibrations at 1638 and 1710 cm^{-1} correspond to AMIDE I carbonyl and coumarin carbonyl, respectively. A broadband at 3388 cm^{-1} corresponds to –OH stretching vibration of serine methyl ester amino acid. Formation of the compound (**IIIe)** was further confirmed by the proton NMR spectrum, wherein the formation of a new amide bond was confirmed by the absence of –COOH signal and appearance of a doublet at δ 9.59–9.57 ppm which corresponds to –NH proton. Further, the molecular ion peak at 276.02 [M + H] in the mass spectrum of the compound confirmed the proposed structure. The physicochemical data of synthesized compounds **III (a–g)** has been displayed in Table S1 (given in Supplementary file).

Biological evaluation

The *in vitro* anti-inflammatory activity of all the newly synthesized coumarin-amino acid conjugates was assessed by two functional models: (a) Protein denaturation by Bovine Serum albumin and (b) Protein Denaturation Egg Albumin Denaturation Method.

The antioxidant efficacy of synthesized compounds was screened by DPPH radical scavenging and Nitric oxide assay.

Anti-inflammatory activity

Protein denaturation by bovine serum albumin method

The potency of the synthesized peptide analogs was evaluated for their anti-inflammatory activity by protein denaturation using Bovine Serum Albumin Method and the outcome of the screening is summarized in Table S2 in the Supplementary file.

The bio-screening profile revealed that all the newly synthesized conjugates showed good to moderate inhibition efficacy. Among the synthesized compounds, phenylalanine conjugate (**IIIf**) showed potent inhibition efficacy compared to standard and compound **IIIc** exhibited good protein denaturation inhibition with minimum inhibitory concentration (36.29). This may be attributed due to the presence of a hydrophobic phenyl group. The compound with tyrosine conjugation (**IIIg**) showed the least activity among the series, it may be due to the presence of phenolic group which is hydrophilic, whereas coumarin coupled with aliphatic amino acids like serine, alanine and glycine showed moderate activity, which may be due to the presence of polar alcoholic and a non-polar alkyl group.

Protein denaturation by egg albumin denaturation method

The anti-inflammatory efficiency of the conjugates was also screened by protein denaturation using Egg Albumin Denaturation Method and the result of the assay is tabulated in Table S3 in the Supplementary file.

It was observed from the table that the highest activity was displayed by the Serine conjugate **IIIe** followed by another conjugate containing leucine amino acid ester **(IIId)** both are aliphatic. Peptide template in which coumarin-3-carboxylic acid is coupled with aromatic amino acids such as phenylalanine (**IIIf**) and valine benzyl ester **(IIIc)** displayed moderate activity. It is quite interesting that the aliphatic amino acid conjugates which showed good activity in egg albumin denaturation method whereas the same showed moderate activity in the bovine serum albumin method. Similarly, the conjugates containing aromatic amino acid segment exhibited potent activity in the BSA method but in the case of egg albumin denaturation, the same conjugates presented weak potency. From both activity profiles, it is clear that the conjugate containing the tyrosine skeleton exhibited poor efficiency toward inhibition of protein denaturation.

Antioxidant activity

It is well documented that free radicals play a major role in the inflammation process since many of the existing non-steroidal drugs were found to act by inhibiting free radical production or radical scavengers.[16] The compounds with anti-inflammatory properties are expected to possess antioxidant activity. Based on these facts we have assessed the synthesized conjugates to their free radical scavenging ability with regard to standard antioxidant agents.

DPPH free radical scavenging activity

DPPH free radical scavenging assay is one of the most widely accepted protocols for screening the ability of synthesized compounds to act as inhibitors/scavengers of free radicals. This assay is based on the formation of stable DPPH free radical in the presence of electron or hydrogen donating antioxidants. The newly synthesized coumarin-amino acid ester conjugates **III (a–g)** were evaluated for their radical scavenging capacity by taking ascorbic acid as standard. All the compounds showed varying degrees of inhibition efficacy and the result is expressed as IC_{50} value (Supplementary Table S4). It is also observed from the table that the scavenging efficiency of each compound increases with an increase in the concentration of test samples.

Among the series, compound **IIIg** and **IIIe** showed potent radical scavenging efficacy with the least IC_{50} value 28.23 and 31.45 µg/mL, respectively, with comparable to the IC_{50} value of standard ascorbic acid (20.53 µg/mL). This may be due to the presence of hydrogen-donating phenolic –OH and the aliphatic hydroxyl group of tyrosine and serine amino acid fragment of target compounds respectively. Further, compound **IIIa** with glycine amino acid conjugation showed poor activity. Compounds **IIIc, IIId** and **IIIb** showed good to moderate radical scavenging property.

Nitric oxide scavenging activity

Nitric oxide radical plays a key role in many biological functions of mammalian and also human body at low concentration. Overexpression of nitric oxide contributes to physiological damages. NO free radical acts cellular messengers and protects some body organs. The synthesized coumarin conjugates were evaluated for their nitric oxide antiradical capacity and the result of the assay are given in Table S5 in the Supplementary file.

Among the synthesized derivatives, significant scavenging activity was shown by the compounds with phenolic and aliphatic –OH groups with the least IC_{50} values comparable with the standard. Similarly, the compound with glycine amino acid conjugation **(IIIa)** showed a declining trend in the activity profile. The rest of the compounds showed good to moderate nitric oxide scavenging property.

Conclusion

A series of coumarinyl-amino acid ester conjugates were synthesized by using acid-amine coupling reagent and appraised for their biological efficiency. From the anti-inflammatory result, it was clear that conjugates with aromatic amino acids showed potent inhibition of protein denaturation of bovine serum albumin whereas aliphatic amino acids showed compelling inhibition of egg albumin protein. The presence of phenolic and aliphatic hydroxyl group in the side-chain functionality of coumarinyl-amino acid ester conjugates showed a declining trend in the activity data. On the other hand, the antioxidant activity results revealed that the presence of different functional groups on the side-chain of amino acid fragment affects the radical scavenging activity such as –OH group in tyrosine and serine exhibited the highest efficiency toward DDPH radical scavenging. Similarly, the electron-donating groups presented as good scavengers of the nitric oxide free radical. Overall, conjugation of the different amino

acid esters with coumarin-3-carboxylic acid was found to exhibit a varying degree of biological potency.

General procedure for coupling of amino acids with 2-oxo-2H-1-benzopyran-3-carboxylic acid

To the cooled solution of 2-oxo-2*H*-1-benzopyran-3-carboxylic acid **I** (1 mmol) in DMF, N-methyl morpholine (1 mmol) was added and stirred for 15 min at 0–5 °C. EDC.HCl (1 mmol) was added to the above solution while maintaining the temperature at 0–5 °C followed by the addition of HOBt (1 mmol) in DMF. The resultant reaction mixture was stirred for additional 10–15 min at the same temperature. To this homogeneous reaction mixture, a pre-cooled solution of esters of amino acids **II (a–g)** (1 mmol) in DMF was added slowly and pH of the solution was adjusted to eight by adding N-methyl morpholine and stirred overnight at room temperature. After completion of the reaction, the whole mass was poured to ice-cold water and stirred for 30 min. The precipitate formed was filtered, dried and recrystallized from ethanol to get the desired products **III (a–g)**.

Ethyl [(2-oxo-2H-1-benzopyran-3-carbonyl) amino] acetate (IIIa)

Yield: 88%; White solid; mp: 171–173 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.16–1.19 (t, J= 12 Hz, 3H, ester-CH_3), 4.09 (s, 2H, CH_2), 7.41–7.48 (q, 2H, Ar–H), 7.71–7.72 (d, J= 4 Hz, 1H, Ar–H), 7.97–7.95 (d, J = 8 Hz, 1H, Ar–H), 8.87 (s, 1H, Ar–H), 9.03 (s, 1H, amide NH). 13C NMR (100 MHz, DMSO-d_6, δ ppm): 14.51 (ester-CH_3), 41.92 (amide-CH_2), 61.01 (ester-CH_2), 116.598, 118.533, 118.826, 125.597, 130.860, 134.772, 148.63 and 154.43 (coumarin C–Ar), 160.73, 161.82 (coumarin –C–O) and 169.86 (glycine ester –C–O). HRMS (ESI): m/z Anal. Calcd: 275.2567; Found: 276.0385 (M + H).

Acknowledgments

The authors thank the Chairman, Department of Industrial Chemistry, Kuvempu University, Shankaraghatta for providing laboratory facilities.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Hadjipavlou-Litina, D.; Kontogiorgis, C.; Pontiki, E.; Dakanali, M.; Akoumianaki, A.; Haralambos, E. K. Anti-Inflammatory and Antioxidant Activity of Coumarins Designed as Potential fluorescent Zinc Sensors. *J. Enz. Inhi. Med. Chem.* **2007**, *22*, 287–292. DOI: 10.1080/14756360601073914.

[2] Suresh, S. K.; Kallappa, M. H.; Arun, K. S. Physicochemical Properties, Antioxidant and anti-Inflammatory Activities of Coumarin-Carbonodithioate Hybrids. *Asian Pac. J. Trop. Biomed.* **2018**, *8*, 201–206.

[3] Esteves, A. P.; Rodrigues, L. M.; Silva, M. E.; Sanjay, G.; Oliveira-Campos, A. M. F.; Machalicky, O.; Mendonc, A. J. Synthesis and Characterization of Novel Fluorescent N-Glycoconjugates. *Tetrahedron* **2005**, *61*, 8625–8632. DOI: 10.1016/j.tet.2005.07.006.

[4] Abha, K.; Sarah, J.; Rakesh, T.; Amir, N. S.; Shilpi, G.; Shiv K., Keykavous, P.; Sunil, K. S. Substituted Coumarin Derivatives: Synthesis and Evaluation of Antiproliferative and Src Kinase Inhibitory Activities. *Chem. Biol. Interface* **2011**, *1*, 279–296.

[5] Lee-Chiang, L.; Chun-Tzu, Y.; Charng-Sheng, T. A CD Exciton Chirality Method for Determination of the Absolute Configuration of Threo-â-Aryl-â-Hydroxy-r-Amino Acid Derivatives. *J. Org. Chem.* **2002**, *67*, 1368–1371. DOI: 10.1021/jo016070l.

[6] Brahmachari, G. Room Temperature One-Pot Green Synthesis of Coumarin-3-Carboxylic Acids in Water: A Practical Method for the Large-Scale Synthesis. *ACS Sustain. Chem. Eng.* **2015**, *3*, 2350–2358. DOI: 10.1021/acssuschemeng.5b00826.

[7] Suhas, R.; Chandrashekar, S.; Channe Gowda, D. Synthesis of Elastin Based Peptides Conjugated to Benzisoxazole as a New Class of Potent Antimicrobials – A Novel Approach to Enhance Biocompatibility. *Eur. J. Med. Chem.* **2011**, *46*, 704–711. DOI: 10.1016/j.ejmech.2010.12.005.

[8] Kumara, H. K.; Suhas, R.; Suyoga Vardhan, D. M.; Shiva Kumar, J.; Channe Gowda, D. Dipeptides as Linker for Multicomponent Presentation—A Facile, Robust, and High-Bioactivity Yielding Strategy. *Med. Chem. Res.* **2018**, *27*, 1504–1516. DOI: 10.1007/s00044-018-2168-y.

[9] Rakesh, K. P.; Ramesh, S.; Kumar, H. M.; Chandan, S.; Gowda, D. C. Quinazolinones Linked Amino Acids Derivatives as a New Class of Promisingantimicrobial, Antioxidant and Anti-Inflammatory Agents. *Eur. J. Chem.* **2015**, *6*, 254–260. DOI: 10.5155/eurjchem.6.3.254-260.1233.

[10] Suhas, R.; Chandrashekar, S.; Channe Gowda, D. Synthesis of Uriedo and Thiouriedo Derivatives of Peptide Conjugated Heterocycles – A New Class of Promising Antimicrobials. *Eur. J. Med. Chem.* **2012**, *48*, 179–191. DOI: 10.1016/j.ejmech.2011.12.012.

[11] Christian, A. G. N. M.; Virginie, F. Amide Bond Formation and Peptide Coupling. *Tetrahedron* **2005**, *61*, 10827–10852. DOI: 10.1016/j.tet.2005.08.031.

[12] Ghanei-Nasab, S.; Khoobi, M.; Hadizadeh, F.; Marjani, A.; Moradi, A.; Nadri, H.; Emam, S.; Foroumadi, A.; Shafiee, A. Synthesis and Anticholinesterase Activity of Coumarin-3-Carboxamides Bearing Tryptamine Moiety. *Eur. J. Med. Chem.* **2016**, *121*, 40–46. DOI: 10.1016/j.ejmech.2016.05.014.

[13] Bouckaert, C.; Silvia, S.; Rondelet, G.; Eduard, D.; Johan, W.; Jean-Michel, D.; Raphaël, F.; Lionel, P. Synthesis, Evaluation and Structure-Activity Relationship of New3-Carboxamide Coumarins as FXIIa Inhibitors. *Eur. J. Med. Chem.* **2016**, *110*, 181–194. DOI: 10.1016/j.ejmech.2016.01.0230223.

[14] Franco, C.; Bruna, B.; Adriana, B.; Daniela, S.; Paola, C.; Arianna, G.; Simone, C.; Daniela, R.; Alessandra, Z.; Maddalena, S. M.; et al. Synthesis, Selective Anti-Helicobacter Pyloriactivity, and Cytotoxicityof Novel N-Substituted-2-Oxo-2H-1-Benzopyran-3-Carboxamides. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4922–4926. DOI: 10.1016/j.bmcl.2010.06.048.

[15] Swayansiddha, T.; Viswajanani, J. S.; Sahu, S. K. Application of Coupling Reagents in Amide Bond Formation. *Int. J. Chem. Pharm. Rev. Res.* **2015**, *1*, 6–9.

[16] Randive, K. H.; Jaishree, V.; Santosh, K. P.; Kumar, P. Synthesis and Biological Evaluation of Novel Coumarin Derivatives as Antioxidant Agents. *Russ. J. Bioorg. Chem.* **2015**, *41*, 324–332. DOI: 10.1134/S1068162015030085.