

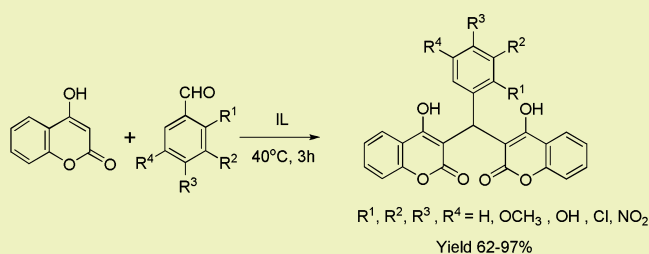
Synthesis of Biscoumarins Using Recyclable and Biodegradable Task-Specific Ionic Liquids

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ABSTRACT: In the frame of research that examines the use of task-specific ionic liquids (ILs) for developing green methodologies toward novel bioactive compounds, ethanolamine-derived ILs were synthesized and used as solvents and catalysts in a domino Knoevenagel–Michael reaction. After extended optimization studies regarding the most important reaction parameters such as reaction time, temperature, and molar ratio of reactants, it was ascertained that the reaction between 4-hydroxy-coumarin and various benzaldehydes in the synthesized ILs proceeded smoothly at 40 °C in 3 h, providing the desired biscoumarins in very satisfactory yields (up to 97%) and high purity after simple workup. The task-specific ILs were recycled and reused three times without a noticeable decrease in their catalytic activity. Moreover, the biodegradability potential of the synthesized ILs has been investigated by applying the biological oxygen demand (BOD-5) closed bottle test. The results indicated that organic carbon was biodegraded up to 59% within five days.

KEYWORDS: Ionic liquids, Domino Knoevenagel–Michael reaction, Biscoumarins, Recyclability, Biodegradability



INTRODUCTION

Green chemistry possesses the spirit of sustainable development and is attracting increasing interest in the 21st century. In the chemical world, strategies for increasing sustainability often require the redesign of reactions and modifications of existing chemical processes aiming, among other things, at the reduction of chemicals used as solvents in a wide range of industrial applications. In this context, ionic liquids (ILs) have recently emerged as a potential replacement for toxic, hazardous, flammable, and highly volatile organic solvents (VOCs). ILs have received a great deal of attention in the synthetic organic chemistry field because of some of their unique properties (thermal and chemical stability, very low vapor pressure, selective solubility of water and organics, recyclability, etc.). Thus, ILs are considered to be a safer alternative to original organic solvents as they are cleaner and safer to use and reuse.^{1,2}

ILs have been increasingly exploited in the pharmaceutical industry in various applications such as drug formulations, solvents for the solubilization of drugs, and for the synthesis of active pharmaceutical ingredients. Therefore, in the frame of research that examines the possibility of using task-specific ILs for developing green methodologies toward novel bioactive compounds, we decided to investigate their application on the synthesis of biscoumarins. Biscoumarins, the bridge-substituted dimers of 4-hydroxycoumarin, have been reported to possess anticoagulant,³ antioxidant,⁴ and enzyme inhibitory^{5–7} activity. The synthesis of biscoumarins is succeeded via a domino

Knoevenagel–Michael reaction between 4-hydroxycoumarin and aromatic aldehydes, and various procedures involving different catalysts and solvents have been reported for this reaction.^{8–12} Recently, the reaction between 4-hydroxycoumarin and substituted benzaldehydes in imidazole-based ILs has been investigated.^{13,14}

In this work, we present the potential of protic ethanolamine-based ILs to carry out the synthesis of biscoumarins under a simple experimental setup and eco-friendly conditions. Moreover, we have also investigated the recyclability and biodegradability of the prepared ILs as one of the most important factors of their environmental fate.

EXPERIMENTAL SECTION

¹H NMR spectra (300 MHz) and ¹³C NMR spectra (75 MHz) were recorded on a Varian Gemini 2000 300 MHz spectrometer. Melting points were determined on a Gallenkamp MFB-595 melting point apparatus and are uncorrected. MS analysis was performed on a Varian 500 MS ion trap mass spectrometer. Instrumental control and data processing are performed by the Varian MS workstation software. The ionization type used is electrospray ionization.

General Procedure for Synthesis of ILs.^{15,16} One equiv of the corresponding carboxylic acid was added dropwise to 1 equiv of ethanolamine at 0 °C, and the reaction mixture was stirred for 24 h at

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room temperature. The produced ILs were dried under high vacuum at 40 °C for 3 h prior to use.

***N*-Hydroxyethylammonium Formate.** Light yellow viscous liquid. ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 8.39 (1H, s, HCOO⁻), 7.72 (4H, br s, -NH₃⁺ and -OH), 3.55 (2H, t, *J* = 5.1 Hz, -OCH₂-), 2.82 (2H, t, *J* = 5.1 Hz, -CH₂-NH₃⁺). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 167.44 (HCOO⁻), 57.95 (CH₂OH), 41.42 (CH₂NH₃⁺).

***N*-Hydroxyethylammonium Acetate.** Light yellow viscous liquid. ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 6.63 (3H, br s, NH₃⁺), 3.50 (2H, br s, HO-CH₂), 2.74 (2H, br s, CH₂NH₃⁺), 1.73 (3H, s, CH₃COO⁻). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 175 (COO⁻), 59.42 (CH₂OH), 42.14 (CH₂NH₃⁺), 23.80 (CH₃).

General Procedure for Synthesis of Biscoumarins 4a–4h. To a round-bottomed flask equipped with a magnetic stirrer were added 4-hydroxy-coumarin (**1**) (2 mmol), the appropriate benzaldehyde (**2**) (1 mmol), and the IL (4 mL). The reaction mixture was stirred at 40 °C under an inert atmosphere. After completion of the reaction that was monitored by TLC, the mixture was cooled at room temperature. H₂O was added, and the formed precipitate was filtered and washed with H₂O. The solid was dissolved in EtOAc, and the solution was washed successively with H₂O and 10% aqueous HCl. The filtrate was also washed with EtOAc (3 times), and the combined organic solutions were dried (Na₂SO₄). The organic solvent was evaporated in vacuo to provide the biscoumarin as a solid. The isolated products were subjected to recrystallization (mixture of hexane and EtOH) to afford analytically pure products. The aqueous filtrate was evaporated in vacuo. The residual IL was dried under high vacuum at 40 °C until constant weight, and thus, it was ready to be reused.

3,3'-(3,4-Dimethoxyphenylmethylene)bis-(4-hydroxy-2H-chromen-2-one) (4a). White solid. Yield 75%. Mp: 257–260 °C (lit.⁹ mp 269–270 °C). ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 17.59 (1H, s, OH), 7.80 (2H, d, *J* = 9 Hz, aromatic H), 7.49 (2H, t, *J* = 9 Hz, aromatic H), 7.26–7.19 (4H, m, aromatic H), 6.74 (1H, d, *J* = 8 Hz, aromatic H), 6.65–6.61 (2H, m, aromatic H), 6.19 (1H, s, CH), 5.14 (1H, br s, OH), 3.68 (3H, s, OCH₃), 3.51 (3H, s, OCH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 168.00, 164.50, 152.47, 148.19, 146.56, 134.93, 130.8, 122.83, 119.98, 118.81, 115.43, 111.53, 103.64, 57.58, 55.45, 35.72. MS (ESI) *m/z* = 495 [M + 23]⁺.

3,3'-(4-Hydroxy-3-methoxyphenylmethylene)bis-(4-hydroxy-2H-chromen-2-one) (4b). White solid. Yield 66%. MP: 252–254 °C (lit. mp¹⁰ 254–259 °C). ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 7.87 (2H, d, *J* = 7.8 Hz, aromatic H), 7.56 (2H, t, *J* = 8.1 Hz, aromatic H), 7.35–7.27 (5H, m, aromatic H), 6.80–6.64 (3H, m, aromatic H, OH), 6.25 (1H, s, CH), 3.69 (3H, s, OCH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 166.68, 164.65, 152.24, 148.44, 146.98, 131.59, 123.89, 123.52, 118.88, 118.38, 115.83, 111.60, 104.21, 55.57, 35.65. MS (ESI) *m/z* = 481 [M + 23]⁺.

3,3'-(2,3-Dimethoxyphenylmethylene)bis-(4-hydroxy-2H-chromen-2-one) (4c). White solid. Yield 62%. Mp: 185–188 °C (lit. mp³ 185–188 °C). ¹H NMR (300 MHz, CDCl₃) δ/ppm: 17.39 (1H, br s, OH), 7.26 (2H, m, aromatic H), 7.15–7.00 (5H, m, aromatic H), 6.83 (1H, m, aromatic H), 6.59 (1H, m, aromatic H), 6.39 (1H, s, CH), 3.24 (3H, s, OCH₃), 3.56 (3H, s, OCH₃). ¹³C NMR (75 MHz, CDCl₃) δ/ppm: 171.69, 168.75, 152.36, 146.01, 134.84, 131.34, 125.12, 123.73, 121.84, 120.30, 119.39, 118.27, 115.88, 110.71, 104.15, 60.13, 55.41, 32.95. MS (ESI) *m/z* = 495 [M + 23]⁺.

3,3'-(4-Chlorophenylmethylene)bis-(4-hydroxy-2H-chromen-2-one) (4d). White solid. Yield 97%. Mp: 255–257 °C (lit. mp⁹ 258–259 °C). ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 17.52 (1H, s, OH), 7.81 (2H, d, *J* = 7.5 Hz, aromatic H), 7.50 (3H, t, *J* = 7.5 Hz, aromatic H), 7.27–7.20 (6H, m, aromatic H), 7.1 (2H, d, *J* = 8.4 Hz, aromatic H), 6.24 (1H, s, CH), 5.20 (1H, br s, OH). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 162.7, 164.63, 152.29, 139.60, 131.81, 130, 128.69, 127.88, 123.95, 123.62, 118.22, 115.90, 103.79, 35.71. MS (ESI) *m/z* = 447 [M]⁺, 449 [M + 2]⁺.

3,3'-(4-Nitrophenylmethylene)bis-(4-hydroxy-2H-chromen-2-one) (4e). White solid. Yield 43%. Mp: 228–232 °C (lit. mp⁹ 238–239 °C). ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 17.38 (1H, s, OH), 8.06 (2H, d, *J* = 8.1 Hz, aromatic H), 7.80 (2H, d, *J* = 8.1 Hz, aromatic H), 7.52 (3H, t, *J* = 8.1 Hz, aromatic H), 7.35 (2H, d, *J* = 8.4 Hz,

aromatic H), 7.29–7.21 (3H, m, aromatic H), 6.34 (1H, s, CH), 5.14 (1H, s, OH). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 167.99, 164.39, 152.63, 145.35, 131.30, 127.93, 124.25, 123.21, 123.12, 119.74, 115.66, 102.80, 36.79. MS (ESI) *m/z* = 458 [M + 1]⁺.

3,3'-(4-Hydroxyphenyl)methylene)bis-(4-hydroxy-2H-chromen-2-one) (4f). White solid. Yield 82%. Mp: 220–222 °C (lit. mp¹⁰ 222–225 °C). ¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 7.87 (2H, dd, *J* = 10.8 Hz, *J* = 1.5 Hz, aromatic H), 7.57 (2H, t, *J* = 8.4 Hz, aromatic H), 7.34–7.27 (3H, m, aromatic H), 6.92 (2H, d, *J* = 8.4 Hz, aromatic H), 6.60 (2H, d, *J* = 8.4 Hz, aromatic H), 6.22 (1H, s, CH). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 167.33, 164.04, 154.22, 132.71, 127.73, 126.91, 124.76, 124.36, 124.16, 116.70, 115.42, 109.39, 105.20, 35.35. MS (ESI) *m/z* = 363 [M - C₅H₅]⁺, 267 [M - 161]⁺.

3,3'-(2,4,5-Trimethoxyphenyl)methylene)bis-(4-hydroxy-2H-chromen-2-one) (4g). White solid. Yield 60%. Mp: 254–257 °C. ¹H NMR (300 MHz, CDCl₃, drops of DMSO-*d*₆) δ/ppm: 8.01 (2H, d, *J* = 8.1 Hz, aromatic H), 7.59 (2H, t, *J* = 6.3 Hz, aromatic H), 7.40–7.34 (3H, m, aromatic H), 6.89 (1H, s, aromatic H), 6.48 (1H, s, aromatic H), 6.08 (1H, s, CH), 3.87 (3H, s, OCH₃), 3.75 (3H, s, OCH₃), 3.54 (3H, s, OCH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ/ppm: 166.61, 164.07, 152.31, 151.83, 147.84, 141.73, 134.53, 130.77, 123.96, 122.97, 119.74, 115.84, 115.49, 104.27, 99.25, 98.65, 56.93, 56.58, 55.84, 32.28. MS (ESI) *m/z* = 525 [M + 23]⁺.

3,3'-(2-Methoxyphenyl)methylene)bis-(4-hydroxy-2H-chromen-2-one) (4h). White solid. Yield 78%. Mp: 218–219 °C (lit. mp³ 214–215 °C). ¹H NMR (300 MHz, CDCl₃, drops of DMSO-*d*₆) δ/ppm: 17.24 (1H, br s, OH), 7.97 (2H, d, *J* = 8.1 Hz, aromatic H), 7.38–7.26 (3H, m, aromatic H), 7.125–7.162 (4H, m, aromatic H), 6.84–6.79 (1H, m, aromatic H), 6.63 (1H, d, *J* = 8.4 Hz, aromatic H), 6.28 (1H, s, CH), 3.37 (3H, s, OCH₃). ¹³C NMR (75 MHz, CDCl₃, drops of DMSO-*d*₆) δ/ppm: 170.86, 170.55, 167.99, 157.32, 152.25, 130.71, 129.59, 129.48, 129.27, 127.02, 124.97, 123.28, 120.33, 115.63, 115.42, 111.46, 103.89, 60.24, 31.00. MS (ESI) *m/z* = 465 [M + 23]⁺.

BOD₅ Test. The method consists of filling with sample to overflowing in an airtight bottle of a specific volume and incubating it at a specified temperature for five days. Dissolved oxygen (DO) is measured initially and after incubation, and the BOD₅ is calculated from the difference between initial and final DO. This difference determines the amount of oxygen that is consumed by suitable populations of microorganisms to metabolize the organic substance.

In particular, triplicate 300 mL bottles containing the IL samples (solution of known IL concentration was added to a nutrient aerobic aqueous medium inoculated with microorganisms) were analyzed for DO concentration at the start and after five days of incubation at 20 °C in the dark. The seed source of microorganisms was mixed liquor that was taken from the activated sludge treatment unit of the central municipal wastewater treatment plant in Athens, Greece. Because less than a 300 mL IL solution was to be analyzed, a specific volume of IL solution was added to a dilution water solution to bring the total sample volume to 300 mL. The dilution water contained appropriate quantities of nutrients and micronutrients that are necessary for the development and action of the microorganisms.

Also, triplicate 300 mL bottles containing a specific volume of the seeding material was added to the dilution water solution to bring the total sample volume to 300 mL, and the initial and final DO concentration was calculated, too. This series referred to as the seed control because the oxygen consumption represents the value of the endogenous oxygen uptake by the microorganisms. In addition, the pH of all samples was maintained in a range from 6.5 to 7.5, which is suitable for bacterial growth.

Finally, triplicate bottles of a third series that contained only dilution water with nutrients (blank solution without microorganisms) were developed, and the values of DO at the start and after five days of incubation were determined. These results were used for the control of the procedure.

The BOD₅ is expressed in eq 1

$$\text{BOD}_5 \text{ (mg/L)} = (D_1 - D_2) - (S_1 - S_2) \times f \times P \quad (1)$$

where *D*₁ is the dissolved oxygen (DO) of the IL diluted sample immediately after preparation. *D*₂ is the DO of the IL diluted sample

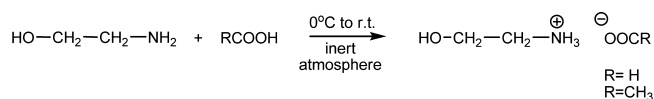
after five days of incubation at 20 °C in the dark. S_1 is the DO of the diluted seed control sample before incubation. S_2 is the DO of the diluted seed control sample after five days of incubation at 20 °C in the dark. f is the ratio of seed in the IL diluted sample/seed in seed control sample. P is the dilution volumetric factor (determined according to the anticipated BOD concentration of the IL sample).

In addition, the following criteria were met: DO depletion in the dilution water blank samples and the seed control samples after five days of incubation were lower than 0.2 mg/L and 1.6 mg/L, respectively. The reduction in DO concentration of the IL samples after incubation were higher than 2 mg/L, and the final DO concentration was higher than 1.5 mg/L. Lower values indicate the shortage or deficiency of population of microorganisms or/and the presence of a high quantity of organic matter.

RESULTS AND DISCUSSION

Two protic ILs, namely, *N*-hydroxyethylammonium formate and *N*-hydroxyethylammonium acetate, were synthesized via a simple acid–base reaction between ethanolamine and the corresponding carboxylic acids (Scheme 1), following a modified literature

Scheme 1. Ionic Liquids Synthesis



procedure.^{15,16} The produced ILs were dried under high vacuum at 40 °C for 3 h prior to use, and no traces of any side products, such as the corresponding amides were observed in their ¹H and ¹³C NMR spectra.

Initially, we studied the reaction between 4-hydroxycoumarin (1) and 3,4-dimethoxy-benzaldehyde (2a) using *N*-hydroxyethylammonium formate as the reaction medium (Scheme 2). Using the reactants at a molar ratio of 1:1 was first examined in order to investigate the possibility of the IL to act as a catalyst for the Knoevenagel condensation between 4-hydroxycoumarin (1) and 3,4-dimethoxy-benzaldehyde (2a) giving us the opportunity to isolate the intermediate product. However, as shown in Table 1 (entries 1 and 2), this reaction provided only a very small amount of the corresponding domino Knoevenagel–Michael product (bis-coumarin 4a), and no traces of the intermediate were detected. Moreover, the starting 4-hydroxycoumarin (1) was detectable at the TLC of the reaction medium even after 3 h and could be recovered after extraction. Therefore, we

Table 1. Optimization Studies Conducted for the Model Reaction between 4-Hydroxycoumarin (1) and 3,4-Dimethoxy-benzaldehyde (2a) Using *N*-Hydroxyethylammonium Formate as Solvent and Catalyst

exp. no	T (°C)	reaction time (h)	coumarin:aldehyde molar ratio	yield % (compound 4a)
1	80	2	1:1	5%
2	40	3	1:1	6%
3	80	24	2:1	9%
4	40	24	2:1	67%
5	40	3	2:1	75%

proceeded to optimization of the reaction conditions aiming at the synthesis of biscoumarins using the molar ratio of 2:1 (Table 1). The temperature of 80 °C was chosen as the starting temperature in order to mimic the reaction conditions using conventional solvents such as ethanol, acetic acid,^{3,4} or even H₂O¹⁰ and other ILs.¹⁴ However, at this temperature, we obtained only a very low yield of the desired biscoumarin 4a, even after 24 h. A possible explanation for this outcome could be that the elevated temperature may favor the formation of the corresponding amide between ethanolamine and formic acid due to dehydration thus lowering the catalytic potential of the IL.

By performing the reaction at 40 °C (Table 1, entries 4 and 5), it was ascertained that the reaction proceeded smoothly using reactants 1 and 2a at a 2:1 molar ratio in 3 h, providing the desired biscoumarin 4a in satisfactory yield (75%) and high purity after simple aqueous workup. After completion of the reaction, the reaction mixture was cooled to room temperature, water was added, and the precipitated solid was filtered off. In order to obtain analytically pure samples, the solid was dissolved in ethyl acetate and washed with water and dilute aqueous hydrochloric acid.

Having established a set of reaction parameters that combine low reaction time with high yield (Table 1, entry 5), we proceeded to examine the scope of the reaction with other substrates (Scheme 2). We were gratified to find that the reaction under these conditions can efficiently provide biscoumarins 4b–4h possessing either electron-donating or electron-withdrawing substituents at various positions of the aromatic ring of the starting benzaldehyde.

In order to investigate the effect of the different anion of the protic IL on the reaction process, we performed some of the reactions using *N*-hydroxyethylammonium acetate at 40 °C for 3h (Table 2).

Scheme 2. Biscoumarins synthesis via a Knoevenagel–Michael Condensation between Aromatic Aldehydes (2) and 4-Hydroxycoumarin (1) in the Presence of Protic ILs

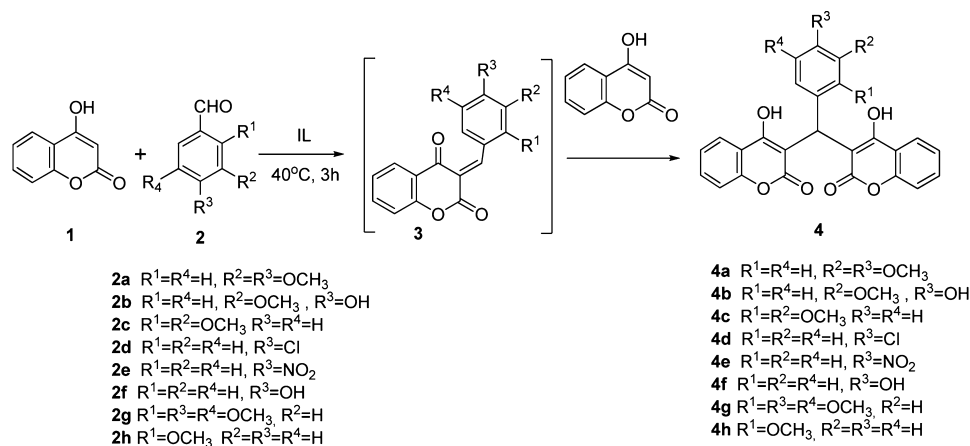
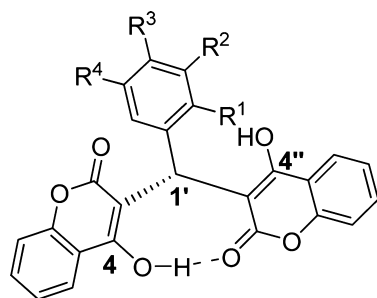


Table 2. Comparison of Reaction Yields of Compounds 4a–4e Using Different ILs

ionic liquid (IL)	product yield (%)				
	4a	4b	4c	4d	4e
HOCH ₂ CH ₂ NH ₃ ⁺ OOCH	75%	66%	84%	77%	43%
HOCH ₂ CH ₂ NH ₃ ⁺ OOCCH ₃	78%	52%	68%	61%	50%

This procedure provided the desired compounds in good yields albeit with lower purity, the main impurity being residual IL.

The structure of the synthesized compounds was unambiguously determined using ¹H and ¹³C NMR spectroscopy and ESI-MS. The presence of a downfield signal, integrating for one proton, indicates that the proton of the hydroxyl group at position 4 of the coumarin moiety takes part in a strong hydrogen bond. This can be explained if we consider the molecule to adopt the conformation shown in Scheme 3. This

Scheme 3. Adopted Conformation of Biscoumarins in DMSO-*d*₆ Solution

observation is in accordance with previous findings for analogous compounds in solution³ and in the crystalline state.¹³

The mass spectra of the synthesized compounds obtained with the ESI technique were characterized by the presence of the molecular ion either as an adduct with sodium (at $m/z = [M + 23]^+$) or an adduct with H⁺ (at $m/z = [M + 1]^+$). In the ESI-MS spectrum of biscoumarin 4d two peaks at $[M]^+$ and $[M + 2]^+$ with an intensity ratio 3:1 were detected, indicating the isotope pattern of chlorine.

The reusability and recyclability of the ILs were then investigated. For this purpose, the same model reaction was again studied under the optimized conditions. After addition of water and filtration of the solid product, the aqueous filtrate containing the IL was washed with ethyl acetate in order to remove any amount of residual product, and then the water was evaporated in vacuo. The residual ionic liquid was dried under high vacuum at 40 °C until constant weight. The structure and purity of the recycled IL were verified with ¹H NMR. This IL was reused for the same reaction up to three times, providing the biscoumarin product in yields and purity comparable to those of the initial reaction.

In order to confidently label this class of solvents as “green”, the biodegradability level of the synthesized ILs was determined applying the biological oxygen demand (BOD₅) closed-bottle test. Biodegradation is the natural process for removal of organic substances from the environment. In general, organic compounds in aquatic environments under aerobic conditions and in the presence of microorganisms can be classified as (1) nonbiodegradable, which means not subjected to any degradation (refractory substances), (2) partially biodegradable, which means biodegraded by microorganisms into byproducts

of a lower molecular weight, resulting in an alteration in their chemical structure, or (3) readily biodegradable, which means rapidly and completely degraded, resulting in the production of carbon dioxide, water, mineral salts, and new microbial cellular constituents (biomass). Consequently, the determination of the biodegradability level of organic substances such as ILs is essential in order to estimate their environmental print and possible negative impact to environment.

Biodegradation tests were carried out according to a standard analytical method to determine the oxygen demand for the biochemical degradation of each organic substance after five days.¹⁷ In general, two stages of degradation take place during the BOD test, carbonaceous and nitrogenous. The carbonaceous stage refers to the conversion of organic carbon to carbon dioxide, while the nitrogenous stage includes the oxidation of organic nitrogen, ammonia, and nitrite to nitrate (nitrification). The entire BOD test starts with the carbonaceous stage, followed by a combination of carbonaceous plus nitrogenous stages. In general, nitrification starts after two to three weeks. When nitrification can occur within five days and the aim is to measure only the carbonaceous oxygen demand, a chemical compound that prevents nitrification is added to the sample, and the results are reported as carbonaceous BOD (CBOD).¹⁸

Taking into account the considerations mentioned above, the following terms are used.

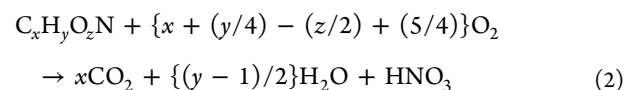
CBOD₅: Oxygen demand by microorganisms for degradation of the organic compound within five days (only carbonaceous stage).

BOD₅: Oxygen demand by microorganisms for degradation of the organic compound within five days, including carbonaceous and nitrogenous stages.

UCBOD: Oxygen demand by microorganisms for the ultimate degradation of the organic compound referring only to the conversion of organic carbon to carbon dioxide, water, and new microbial cellular constituents (ultimate carbonaceous demand).

UBOD: Oxygen demand by microorganisms for the ultimate degradation of the organic compound indicating the level of degradation achieved when the compound is totally used in the production of carbon dioxide, water, mineral salts, and new microbial cellular constituents (ultimate carbonaceous and nitrogenous demand).

UCBOD coincides with the theoretical oxygen demand (ThOD) for complete conversion of the organic carbon to carbon dioxide, while UBOD reflects the ThOD for the complete conversion of the organic carbon to carbon dioxide plus the complete nitrification of the reduced nitrogen forms (organic nitrogen, ammonia, and nitrite) to nitrate. For the ILs under examination, the UBOD should be calculated according to eq 2. The contribution of organic nitrogen in UBOD can be estimated by the theoretical amount of oxygen that is demand for the formation of HNO₃. Finally, the biodegradability level of the organic substance can be calculated and expressed by the ratios BOD₅/UBOD, CBOD₅/UBOD, and CBOD₅/UCBOD.



During the development of the BOD₅ tests of ILs, a small amount of allyl thiourea (ATU) was added to the samples in order to inhibit the activity of nitrifying bacteria. As a result, the oxygen that is used by the nitrification process was not included

Table 3. Values of UBOD, UCBOD, CBOD₅, and CBOD₅/UCBOD of the ILs

ionic liquid (IL)	UBOD (mg/L)	UCBOD (mg/L)	CBOD ₅ (mg/L)	CBOD ₅ /UCBOD
HOCH ₂ CH ₂ NH ₃ ⁺ OOCH ⁻ (initial concentration: 200 mg/L)	299.1	209.4	123.3	58.9
HOCH ₂ CH ₂ NH ₃ ⁺ OOCCH ₃ (initial concentration: 216 mg/L)	371.3	285.6	141.3	49.5

Table 4. Theoretical Oxygen Demand (mg/L) for Degradation of Each IL Molecule as a Whole and Partial

ionic liquid (IL)	UBOD cation [C ₂ H ₈ ON] ⁺	UCBOD cation [C ₂ H ₈ ON] ⁺	UCBOD [CH ₃ O]	UBOD [CH ₃ N]	UCBOD [CH ₃ N]	UCBOD anion ⁻ [OOCH]
HOCH ₂ CH ₂ NH ₃ ⁺ OOCH (initial concentration: 200 mg/L)	284,4	195,3	74,8	209,6	120,5	14,9
HOCH ₂ CH ₂ NH ₃ ⁺ OOCCH ₃ (initial concentration: 216 mg/L)	272,1	186,4	71,6	200,5	114,8	99,9

in the obtained results, and the consumed oxygen refers only to CBOD₅. Taking into consideration the measured CBOD₅ as obtained by eq 1 and the quantities of ILs used in CBOD₅ tests in combination with the stoichiometry of the eq 2, the values of CBOD₅, UBOD, UCBOD, and the ratio CBOD₅/UCBOD per IL were calculated and are presented in Table 3. The experimental results indicated that the ILs under examination present remarkable biodegradability potential because a percentage of about or more than 50% of the organic carbon was biodegraded within five days (58.9% and 49.5%, respectively).

In order to obtain an insight on which part of the IL is subjected to biodegradation more easily, a theoretical approach was followed. In particular, the theoretical oxygen demand for the degradation of the cation (as a whole and partially) and anion of each IL molecule was calculated (Table 4) and compared to the measured CBOD₅.

By comparing the data of Table 4 with the experimental results, it is observed that the measured CBOD₅ for the two ILs are (1) extremely higher than the UCBOD of the anion, (2) lower than the UCBOD of the cation as a whole, (3) much higher than the UCBOD of the part of the cation that contains the hydroxyl [CH₃O], and (4) slightly higher than the UCBOD of the part of the cation that contains the nitrogen [CH₃N].

The quantitative and qualitative data mentioned above indicate that the biodegradability level of the ILs during the time period of five days is due to the complete degradation of the part of the cation that contains the hydroxyl group [CH₂OH] and the partial degradation of the remaining part of the cation [⁺H₃NCH₂] (oxidation of nitrogen did not occur). These conclusions are supported by literature data, and the biodegradability level depends mainly on the cation of the IL molecule and on the groups that provide possible sites for enzymatic hydrolysis, especially oxygen atoms (e.g., in the form of hydroxyls) that present high degradation potential.^{19–22}

In conclusion, the ability of ethanolamine-based ILs to act as solvents and catalysts for a domino Knoevenagel–Michael condensation in order to synthesize biscoumarins has been investigated. These ILs that act as alternative solvents and catalysts are cheap, rapidly and easily prepared, satisfactory biodegradable, recyclable, and not harmful to the environment compared to conventional solvents. The final products were obtained in high purity and in satisfactory yields, comparable or better than the corresponding yields produced by heating in conventional solvents (EtOH or CH₃COOH), which range from 50% to 87%.^{3,4} In addition to the purity of the products, the short reaction times and ease of workup make the method advantageous. The developed methodology is environmentally friendly with green chemistry credentials as the ILs can be recycled and reused while they present remarkable biodegradability potential in a short time period.

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Notes

The authors declare no competing financial interest.

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