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Glycerol and acetic acid assisted mild strategy for facile synthesis of 3-heteroarylcoumarins via three-component reaction

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Abstract

A feasible and inexpensive reaction system for synthesis of 3-benzoxazol-2-yl-chromen-2-one as a kind of 3-heteroarylcoumarins has been developed. Both acetic acid and glycerol are simple, cheap and common chemicals, the combination of them could smoothly accelerate the three-component one-pot reaction of salicylaldehyde, *o*-aminophenol and ethyl cyanoacetate to yield 3-heteroarylcoumarins. This process not only offers the products in good to excellent yields but also avoids the problems associated with catalyst cost, handling, safety and pollution. Compared with the literature, the reaction temperature successfully reduced to 80 °C. As an application, disperse yellow 232 was furnished with a satisfactory yield under optimum reaction conditions. In addition, all synthesized 3-heteroarylcoumarins were preliminary evaluated the inhibitory effect against human carboxylesterase 1. This protocol offers an expedient strategy for efficient synthesis of 3-heteroarylcoumarins that are widely present in biologically active compounds and fluorescent paints.

Keywords 3-Benzoxazol-2-yl-chromen-2-one · Three-component reaction · Mild synthesis · Glycerol · Acetic acid

Introduction

Coumarin (2-oxo-2*H*-1-benzopyran) scaffold has attracted considerable attention from chemist due to be a "privileged" unit in lots of natural products and synthetic organic compounds [1–11]. The 3-heteroarylcoumarins containing benzoxazole moiety exhibits broad optical properties and important biological activities [12–17]. In addition to coloring polymers, they are also particularly useful in a wide range

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such as fluorescent paint and resins for solar collectors, sensitive material and optical disk recording materials [18–22]. As shown in Fig. 1, 3-benzoxazol-2-yl-chromen-2-one is an available drug in the market and 3-(5-chlorobenzo[*d*]oxazol-2-yl)-7-(diethylamino)-2*H*-chromen-2-one is a famous dye called disperse yellow 232. At present, the reported synthetic methodologies of them mainly include multi-step synthesis procedures and convergent synthesis [23–26]. These methods typically require several steps, use expensive reagents, employ complex catalytic systems and require the preparation of raw materials in advance, which adds complexity to the operation and often reduces yields [25, 27]. Although some improvements for the synthesis of 3-heteroarylcoumarins have been reported, the versatile and efficient methodology to straightforward construction of them from ordinary commercial materials continues to pose a challenge [25].

Multi-component reactions (MCRs) are faster and cheaper than traditional approach, since the reaction will be completed by just mixing simple starting materials together in a single vessel without isolating any intermediate [28–30]. This strategy has been recognized as powerful and responsible tool for creating novel and complex organic molecules [31–36]. However, only a few reports describe the synthesis of 3-benzoxazol-2-yl-chromen-2-one via three-component one-pot method using ZSM-5 [19], benzoic acid as catalyst [37, 38], or under catalyst free condition [27]. The yields of these reactions are not completely satisfactory, the reaction temperature is still high or solvent is not easy to remove. One of our efforts in recent years is the development of mild and efficient methodologies for the synthesis of coumarin derivatives [39–41]. Following this aim, we recently demonstrated that low-transition-temperature mixture (LTTM) which formed from *L*(-)-Proline and oxalic acid could smoothly promote the title reaction [42]. In that work, we noted that oxalic acid offered a moderate yield of the target product. Although the yield did not increase when the subsequent conditions were optimized, we still think using a common and simple organic acid as catalyst has much room for further development. Glycerol has been used as single solvent, co-solvent, part of deep eutectic mixture, or playing the double role of solvent and reagent. Performing reaction in glycerol not only offers a high reaction yield, but also avoids the generation of toxic wastes, the use of large amount of organic solvents and tedious post-treatment [43, 44]. Continuously developing alternative and efficient approaches for construction of 3-benzoxazol-2-yl-chromen-2-one will be an important topic in our group. Here, an attractive strategy for synthesis of target compound reacted in glycerol and accelerated by a common and simple organic acid is developed.

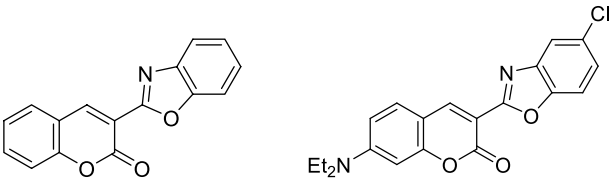


Fig. 1 Structure of 3-benzoxazol-2-yl-chromen-2-one and its derivative disperse yellow 232

Experimental

General

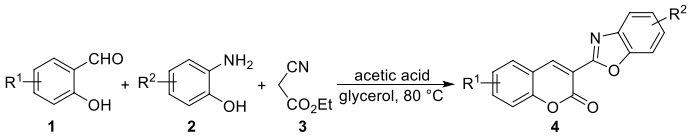
Unless otherwise stated, all reagents were obtained from commercial sources and were used without further purification. Melting points were measured on a Beijing Tech X-5 melting point detector and were uncorrected. The IR spectra were recorded with a Bruker Shimadzu IR-460 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were determined on a Bruker Avance III 500 MHz or Bruker Avance NEO 600. The chemical shifts (δ) were reported in parts per million (ppm) and coupling constants (J) in Hertz. The substrate of CES1, N-alkylated D-luciferin methyl ester (NLMe) was synthesized in our laboratory [45]. Human liver microsomes (HLM from 50 donors, lot X008067) were obtained from Bioreclamation IVT. Bis-p-nitrophenyl phosphate (BNPP) was purchased from TCI. Luciferin detection reagent (LDR) was purchased from Promega Biotech. Substrate such as 3-heteroarylcoumarins 4, NLMe was dissolved in DMSO and stored in a refrigerator at 4 ℃ until use. Phosphate buffered saline (0.1 M, pH 6.5) stored at 4 °C. Millipore Water and LC grade acetonitrile were used for all experiments.

Typical experimental procedure for synthesis of 3-(benzo[d] oxazol-2-yl)-2H-chromen-2-one

The main synthetic root is described in Scheme 1. A mixture of salicylaldehyde (0.50 mmol), *o*-aminophenol (0.60 mmol) and ethyl cyanoacetate (0.55 mmol) was placed in a 10-mL Schlenk tube, and then, 1 mmol acetic acid and 0.2 mL glycerol were added. The reaction mixture was stirred at the indicated temperature for 14 h. After completion of the reaction, a solution of Na_2CO_3 and ethanol were added to the mixture and vigorously stirred for a while. Finally, the precipitate was filtrated and washed with 50% ethanol without further purification to afford the corresponding pure compound.

CES1 inhibition assay

The total volume of the whole system was 100 μL, including the following components: 2 μL DMSO/inhibitor, 91 μL buffer PBS (pH = 6.5), 5 μL human liver microsomes (final concentration 1 μg/mL), 2 μL Substrate NLMe. Step 1: Dissolved 3-heteroarylcoumarins **4** in DMSO into 0.05, 0.5, and 5 μM solutions, respectively (final concentrations 1, 10, 100 μM, respectively). Step 2: The experiment set up three parallel groups, one is the experimental group, one is the non-inhibitor DMSO group, and the other



Scheme 1 Synthesis root of 3-benzoxazol-2-yl-chromen-2-one

is the blank group without HLM. In the experimental group, 2 μL of the experimental inhibitor of the corresponding concentration was added to the 96-well plate, and then 91 μL of PBS and 5 μL of HLM were added. As the control group, added 2 μL of DMSO to a 96-well plate, and then added 91 μL of PBS and 5 μL of HLM. The blank group without HLM was used as a fluorescence control for the decomposition of the substrate itself. The added substance did not contain 5 μL of HLM, and it could be filled with 5 μL of PBS. The added substances were 2 μL of inhibitor and 96 μL of PBS. Step 3: After incubating the 96-well plate for 3 min, added 2 μL D3 of the substrate, and incubated for another 10 min. After the incubation was completed, added 50 μL to the white plate containing 50 μL of LDR, sent it to the microplate reader for detection, and placed it in the automatic fluorescence reading plate for chemiluminescence analysis. Step 4: Residual activity (%) = (Fluorescence intensity in the presence of inhibitors-Blank fluorescence intensity of each experimental group)/[Fluorescence intensity of the negative control (DMSO only)-Blank of the experimental group in the presence of each inhibitor fluorescence intensity] × 100%.

Results and discussion

Here, the main concern of this work was making present synthetic strategy with more reasonable yields. Salicylaldehyde (0.50 mmol), *o*-aminophenol (0.55 mmol) and ethyl cyanoacetate (0.55 mmol) were selected as model substrates for optimization of the reaction parameters. Primarily, three common and simple organic acids were chosen to promote this reaction (Table 1, entries 1–3). The amount of oxalic acid, citric acid and acetic acid is determined according to the number of their carboxyl groups. Acetic acid offered a slight higher yield. So, more common and cheap acetic acid was employed as promoter. Also because acetic acid was liquid which was more easily to blend with starting materials to a homogenous state. Further screening showed that two equivalent of acetic acid was sufficient to promote reaction and the yield could not be improved by adding more acetic acid (Table 1, entry 5). As expected, ethylene glycol provided similar result in comparison to glycerol. But ethanol gave lower yield than both of them (Table 1, entries 7, 8). Glycerol is produced in large amounts in biodiesel production process. It is now a readily available and cheap resource. The ethylene glycol has a relatively higher price. As a result, glycerol turned into our selection. The volume of glycerol was also explored and 0.2 mL became the best choice. Testing material ratio in detail found that a satisfied result was obtained with 1:1.2:1.1 molar ratio of salicylaldehyde, *o*-aminophenol and ethyl cyanoacetate (Table 1, entry 12). Eventually, the effect of temperature and reaction time was also investigated. It was found that increasing reaction temperature to 90 ℃ could not improve the yield (Table 1, entry 11). The optimal reaction duration was 14 h and the yield could get as high as 97%. From above, the best result was achieved.

In order to demonstrate the scope of this strategy, various salicylaldehydes having different functional groups and *o*-aminophenol were employed. Satisfactorily, these reactions could take place easily and afford corresponding compounds with good yields. However, no matter electron-donating or -withdrawing group on

Table 1 Screening of reaction conditions

Entry[a]	Catalyst (eq)	Solvent	Volume/mL	Temp./°C	Time/h	Yield[b]/%
1	oxalic acid (0.3)	Glycerol	0.1	80	9.5	50
2	Citric acid (0.2)	Glycerol	0.1	80	9.5	51
3	AcOH (0.6)	Glycerol	0.1	80	9.5	54
4	AcOH (1.0)	Glycerol	0.1	80	9.5	63
5	AcOH (2.0)	Glycerol	0.1	80	9.5	82
6	AcOH (2.5)	Glycerol	0.1	80	9.5	81
7	AcOH (2.0)	Ethylene glycol	0.1	80	9.5	80
8	AcOH (2.0)	Ethanol	0.1	80	9.5	70
9	AcOH (2.0)	Glycerol	0	80	9.5	70
10	AcOH (2.0)	Glycerol	0.2	80	9.5	87
11	AcOH (2.0)	Glycerol	0.2	90	9.5	89
12[c]	AcOH (2.0)	Glycerol	0.2	80	9.5	91
13[c]	AcOH (2.0)	Glycerol	0.2	80	14	97

[a] Salicylaldehyde (0.50 mmol), *o*-aminophenol (0.50 mmol) and ethyl cyanoacetate (0.55 mmol)

[b] Isolated yield

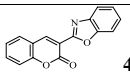
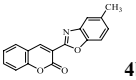
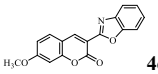
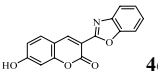
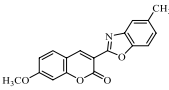
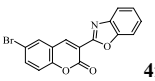
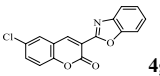
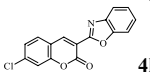
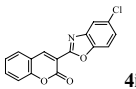
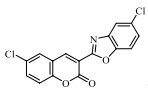
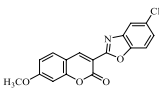
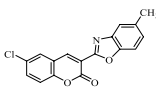
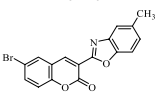
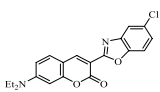
[c] Salicylaldehyde (0.50 mmol), *o*-aminophenol (0.60 mmol) and ethyl cyanoacetate (0.55 mmol)

the salicylaldehyde, the yield was lower than model reaction (Table 2). Relatively higher yields of products were obtained for *o*-aminophenol bearing an electron-donating group (**4b**, **4d**, **4k**, **4l**). Disperse yellow, as we all know, is a class of functional dyes with strong fluorescence. We were stimulated by above success, the versatility of this reaction was explored further by extending the procedure to synthesis of dispersed yellow 232 molecules (**4n**). A 75% yield of disperse yellow 232 was observed which is comparable to the reported result. Overall, this strategy is general and has broad substrate scope.

Up to now, only few articles reported the synthesis of this type of compounds via three-component one-pot pathway and some of the method employ harsh reaction conditions. Taking **4a** as example (Table 3), our major strengths lie in offering higher product yield and successfully reduce the reaction temperature to 80 ℃. Choosing green and common acetic acid as promoter and glycerol as solvent has advantage of that the products could be separate via simple filtration and washing with aqueous ethanol.

Human carboxylesterase 1 (CES1), primarily expressed in the liver and adipocytes, plays key roles in the hydrolysis of endogenous esters (such as cholesteryl esters and triacylglycerols) and the metabolism of xenobiotic esters (such as clopidogrel and oseltamivir), thus participates in physiological and pathological processes [46]. CES1 is currently considered to be a therapeutic target for hypertriglyceridemia. However, only one CES1 inhibitor termed GR148672X has been in preclinical development for the hypertriglyceridemia treatment and no CES1 modulators approved as medicines to date [47, 48]. Thus, N-alkylated D-luciferin methyl ester (NLMe) used as the substrate probe for CES1 [45], a further survey

Table 2 Substrate scope of condensation cyclization of salicylaldehydes, *o*-aminophenol and ethyl cyanoacetate

Entry[a]	Product	Yield[b]/%	Mp/°C
1	 **4a**	97	185~187 (186~189[27])
2	 **4b**	96	166~169 (161~163[27])
3	 **4c**	91	192~194 (191~193[27])
4	 **4d**	87	>300 (314~315[24])
5	 **4e**	95	237~238 (197~199[27])
6	 **4f**	84	240~242 (229~231[27])
7	 **4g**	92	233~235 (237~239[27])
8	 **4h**	78	247~249 (280~282[38])
9	 **4i**	90	239~241 (259~260[27])
10	 **4j**	83	261~263 (258~260[38])
11	 **4k**	80	234~236 (234~236[27])
12	 **4l**	97	232~234 (225~227[27])
13	 **4m**	93	243~245 (248~251[27])
14	 **4n**	75	237~238 (205~208[27])

of compounds **4** was carried out for preliminary screening of their inhibitory effect against CES1 using three inhibitor concentrations (1 μM, 10 μM and 100 μM). It is evident from Fig. 2 that most of tested compounds displayed good inhibitory effect on CES1 at the concentration 100 μM.

Table 3 Comparison of different conditions for the synthesis of **4a**

Entry	Catalyst	Solvent	Conditions	Time (h)	Yield (%)	Year[Ref.]
1	Benzoic acid	*n*-butanol	Refluxed	7	77	2009[38]
2	Catalyst free	*n*-butanol	118 ℃	7	61	2015[27]
3	LTTM	ethanol	85 ℃	12	90	Previous work
4	Acetic acid	glycerol	80 ℃	14	97	Present work

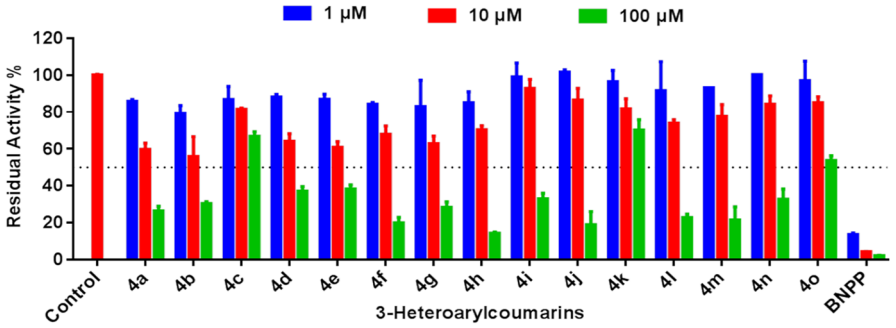


Fig. 2 Inhibition potency of 3-heteroarylcoumarins 4 toward CES1. Bis-p-nitrophenyl phosphate (BNPP) as a positive inhibitor against carboxylesterases

Conclusions

In summary, a convenient three-component one-pot protocol for the synthesis of 3-benzoxazol-2-yl-chromen-2-one using salicylaldehyde, *o*-aminophenol, and ethyl cyanoacetate as raw materials was developed and improved. A combination of acetic acid and glycerol which are simple, cheap and common chemicals could smoothly promote the reaction. This strategy had good functional group tolerance and a series of 3-heteroarylcoumarins could be synthesized in good to excellent yields under mild conditions. Compared with other literatures, we successfully reduced the reaction temperature to 80 °C and simplified the post-treatment procedure. As an application, disperse yellow 232 was obtained in a satisfactory yield through optimum reaction conditions. Furthermore, all synthesized 3-heteroarylcoumarins were preliminary evaluated the inhibitory effect against CES1, which demonstrated moderate inhibitory activity. Other biological activity studies for the 3-heteroarylcoumarins are currently ongoing in our laboratory.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11164-021-04464-0.

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