Article

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Diversity-Oriented Synthesis of Coumarin-Linked Benzimidazoles via a One-pot, Three-step, Intramolecular Knoevenagel Cyclization

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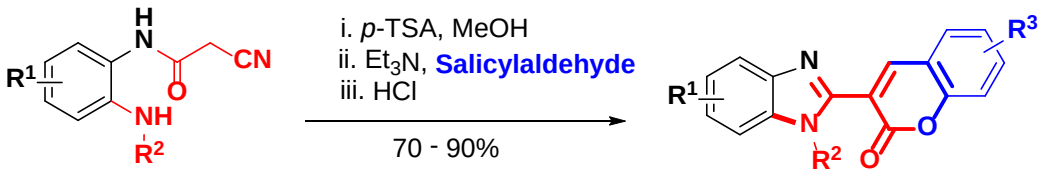
Graphical abstract



● One-pot; ● High Yields; ● Mild Conditions; ● Broad Substrate Scope; ● pkDAO Inhibitor

Abstract

Diversity-oriented synthesis of coumarin-linked benzimidazoles from *N*-(2-aminophenyl)-2-cyanoacetamide was achieved *via* a one-pot, three-step sequential reaction in excellent yields. *In situ* intramolecular cyclization of the cyanoacetamide afforded benzimidazoles which subsequently underwent a Knoevenagel condensation of the 2-cyanomethylbenzimidazoles with salicylaldehydes promoted by triethylamine to reach the target compounds. An important intermediate, 2-(2-imino-2*H*-chromen-3-yl)-1*H*-benzimidazole was characterized by X-ray analysis and further hydrolyzed to 2-(coumarin-3-yl)benzimidazole

in acidic condition. Among the synthesized compounds, some were found to be promising inhibitors of porcine kidney D-amino acid oxidase (*pk*DAO).

KEYWORDS: *Knoevenagel condensation, coumarin, diversity-oriented synthesis, one-pot synthesis, pkDAO activity.*

INTRODUCTION

Diversity-oriented synthesis (DOS) provides direct access to diverse and complex molecular scaffolds. Diversified benzimidazoles linked with a classical heterocyclic moieties, such as pyridine, pyrimidine, thiazole and imidazole have been reported in the literature with significant biological activities.[1,2] For instance, thiabendazole (**I**) is used as an antifungal and antihelminthic agent,[3] pimobendan (**II**) is a selective poly(ADP-ribose)polymerase-1 (PARP) inhibitor and cardiovascular agent,[4] Lansoprazole is a proton-pump inhibitor which prevents the stomach's production of gastric acids[5]. In addition, coumarin derivatives such as Warfarin and Dicoumarol are well-known anti-coagulants[6] and Novobiocin (**III**) is used as a potent antibiotic against Gram-positive bacteria[7]. Tsay and coworkers[8] reported the synthesis of 3-(5-methylbenzimidazol-2-yl)benzocoumarin (**IV**) with anti-hepatitis C virus (HCV) activity (Figure 1).

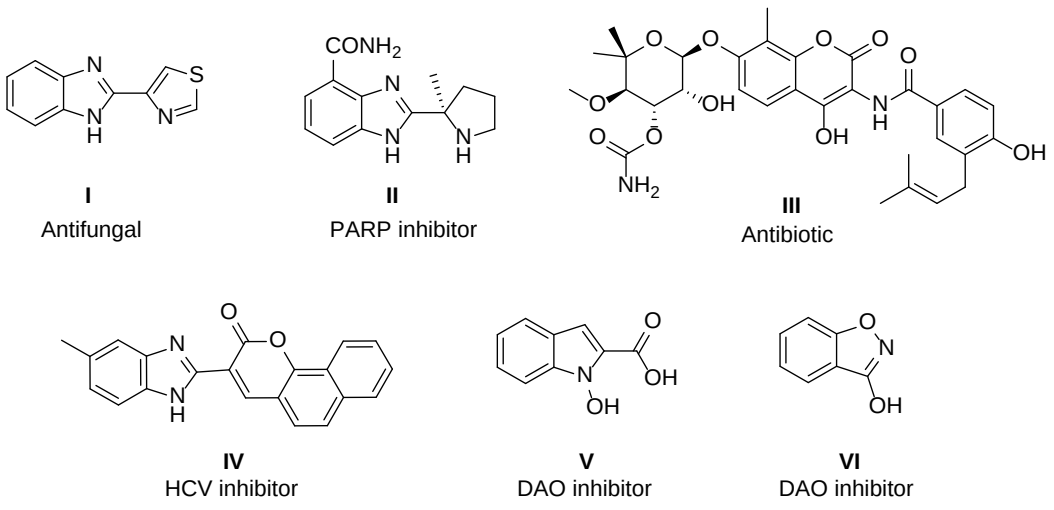


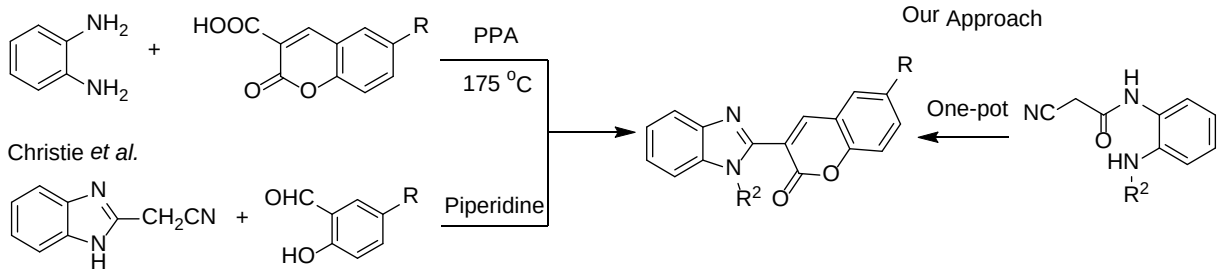
Figure 1. Some bioactive azole and coumarin heterocycles.

D-Amino acid oxidase (DAO) is a stereospecific degradative enzyme for D-amino acids in

mammalian tissues. DAO inhibitors help to increase the D-serine levels in the serum and cerebrospinal fluid of schizophrenic patients.[9-10] In the literature, benzopyrrole[11] (**V,** IC_{50}=1.34 μM) and benzoxazole[12] (**VI,** IC_{50}=1.88 μM) derivatives were found as the human DAO inhibitors. In continuation of our interest to synthesize biologically important heterocycles,[13-14] we wish to report herein an efficient method for the synthesis of coumarin linked benzimidazoles under mild conditions and their evaluation of initial *pk*DAO activity.

Romieu *et al.* reported the synthesis of 3-(2-benzimidazolyl)-7-hydroxycoumarins with only 19-24% yields.[15] Very recently, Wet-osot and coworkers demonstrated the use of *N*-acylbenzotriazole to synthesize 3-arylcoumarins.[16] In 2014, Arora *et al.* reported the synthesis of 2-(coumarin-3-yl)benzimidazoles as an anti-inflammatory agent by condensing coumarin-3-carboxylic acid with *o*-phenylenediamine in the presence of PPA at 175 ^{o}C.[17] Christie and Lui synthesized the same heterocycle from 2-cyanomethyl benzimidazole and salicylaldehyde in the presence of piperidine (Scheme 1).[18]

Scheme 1. Literature reports and our approach for the synthesis of (coumarin-2-yl)benzimidazoles

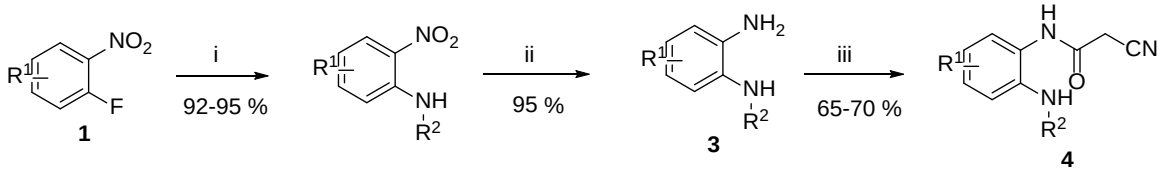


Since the reported syntheses suffers from drawbacks of the multi-step synthesis, use of strong acidic conditions, high temperature and low yields, we planned to achieve a one-pot, three-step protocol for the synthesis of 2-(coumarin-3-yl)benzimidazoles under mild conditions in higher yields.

RESULTS AND DISCUSSION

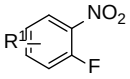
As shown in Scheme 2, the substituted diaminobenzene **3** acted as the first point of structural diversity with various alkyl groups. This diaminobenzene **3** was synthesized from the reaction of substituted *o*-nitrofluorobenzene **1** with amines **2** and successive reduction of nitro group by zinc /ammonium formate in methanol. The regioselective condensation of the primary amine of **3** with cyanoacetic acid was performed in the presence of EDCI and hydroxybenzotriazole (HOBt) in dichloromethane leading to the formation of *N*-(2-aminophenyl)-2-cyanoacetamide **4** (procedure in SI).

Scheme 2. Preparation of *N*-(2-aminophenyl)-2-cyanoacetamides (4)



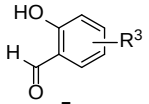
Reaction Conditions: i) R^2NH_2 (**2**), DCM, 40 ^oC, 6h; ii) Zn/$HCOONH_4$, MeOH, rt, 15 min.; iii) Cyano acetic acid, EDCI, HOBt, CH_2Cl_2, 0 ^oC, 30 min, rt 12h;

Figure 2 shows the variety of substituted *o*-nitrofluorobenzenes **1**{*1-3*}, amines **2**{*1-8*} and salicylaldehydes **5**{*1-5*} used in the synthesis.



1

Compound	R_1
1{*1*}	H
1{*2*}	4-$COOCH_3$
1{*3*}	5-Cl



5

Compound	R_3
5{*1*}	H
5{*2*}	4-OCH_3
5{*3*}	5-CH_3
5{*4*}	5-Cl
5{*5*}	5-NO_2

R^2–NH_2

2

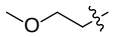
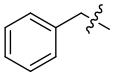
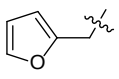
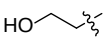
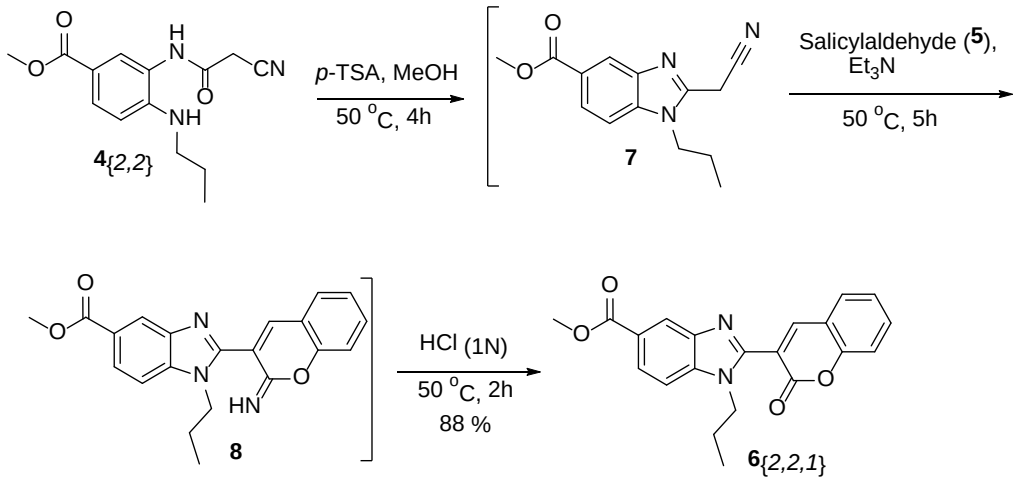
Compound	R_2
2{*1*}	
2{*2*}	
2{*3*}	
2{*4*}	
2{*5*}	
2{*6*}	
2{*7*}	
2{*8*}	

Figure 2. Diversity elements, chemsets **1**{*1-3*}, **2**{*1-8*} and **5**{*1-5*}

Compounds **4** were treated as the common building block for the synthesis of substituted

2-(coumarin-3-yl)benzimidazoles. Methyl 3-(2-cyanoacetamido)-4-(propylamino)benzoate **4**{*2,2*} was chosen as the model substrate for the next step. Conventional strong Brønsted acids viz. TFA, *p*-TSA, HCl etc. were used for the dehydrative cyclization reactions.[19] The dehydrative intramolecular cyclization reaction of **4** proceeded smoothly in the presence of *p*-TSA in methanol at 50 ^{o}C within 4h and the 2-cyanomethyl benzimidazoles formed *in situ* were demonstrated to undergo nucleophilic addition with salicylaldehydes in the presence of triethylamine to yield intermediate **8** in 5h. Further addition of HCl to the same reaction mixture and stirring for 2h afforded the final product **6**{*2,2,1*} with 88% yield (Scheme 3). Generally organic bases[20] such as piperidine, triethylamine (TEA), pyridine, etc. are used for the Knoevenagel condensation, but piperidine and pyridine resulted in lower yields, 76% and 54%, respectively, as compared to TEA (88%).

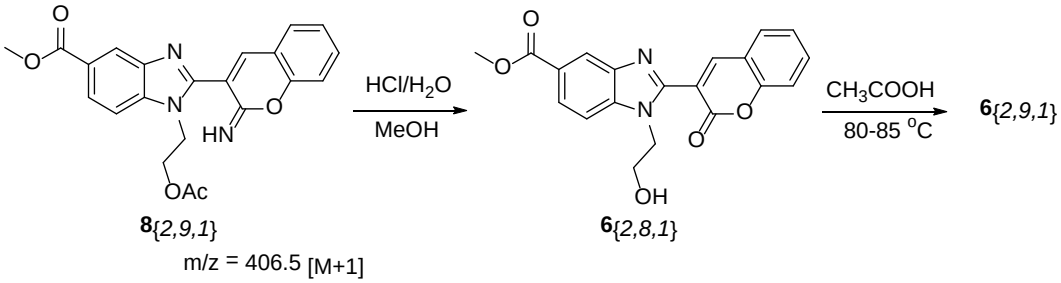
Scheme 3. Synthesis of 2-(coumarin-3-yl) benzimidazoles from *N*-(2-aminophenyl)-2 -cyanoacetamide



This one-pot, three-step process entails the intramolecular cyclization of *N*-(2-aminophenyl)-2-cyanoacetamides **4** to give benzimidazole *in situ* in the presence of *p*-TSA, followed by Knoevenagel condensation and acidic hydrolysis. A variety of alkyl and aralkyl groups viz. propyl, methoxyethyl, cycloalkyl, benzyl, furyl etc. are well tolerated in diversified positions of benzimidazoles (Table 1). Introduction of both electron-withdrawing (6-NO_2 or 6-Cl) as well as electron-donating group (6-Me or 7-OMe) on coumarin yielded the products with good

yields (20 examples, 75-96 %). The compound **6**{*2,9,1*} was prepared by the acetylation of **6**{*2,8,1*} in the presence of acetic acid at 80-85 ^{o}C for 1h.(Scheme 4)

Scheme 4. Preparation of 7{*2,8,1*} **and 7**{*2,9,1*}.



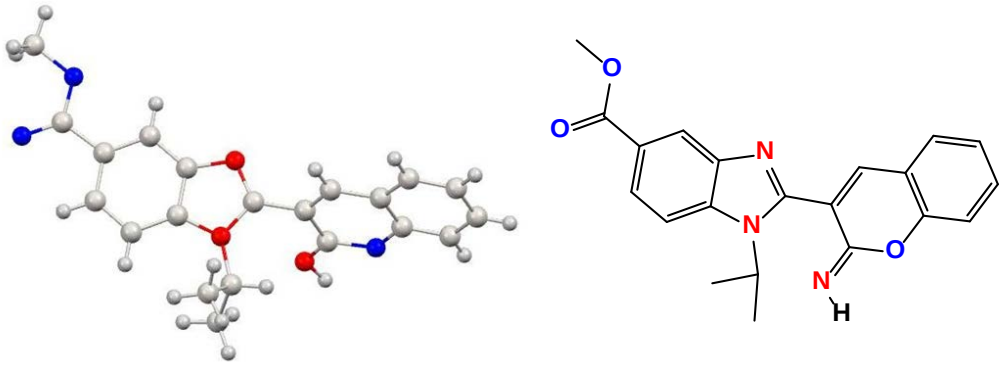
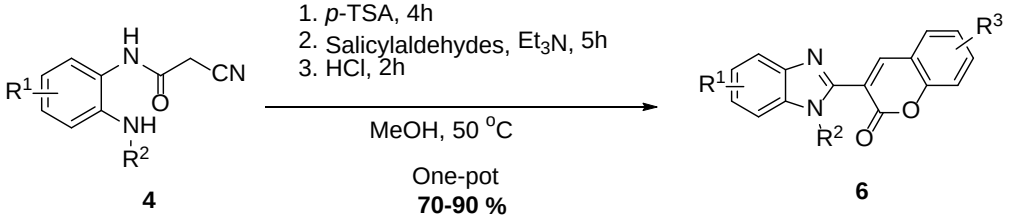
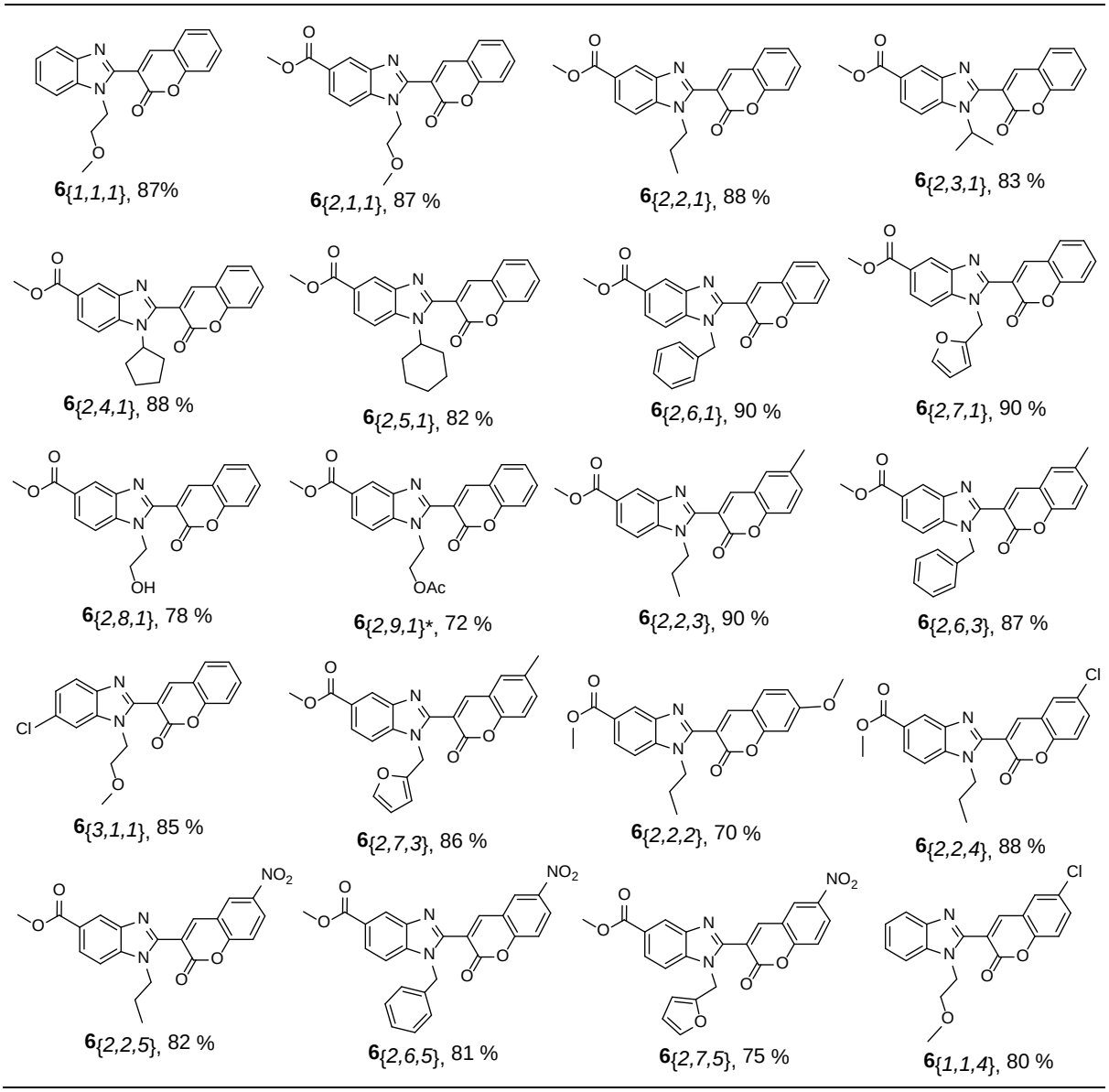


Figure 3. X-Ray crystal structure of **8**{*2,3,1*}.

Table 1. Substrate Scope for the Synthesis of 2-(Coumarin-3-yl)benzimidazoles



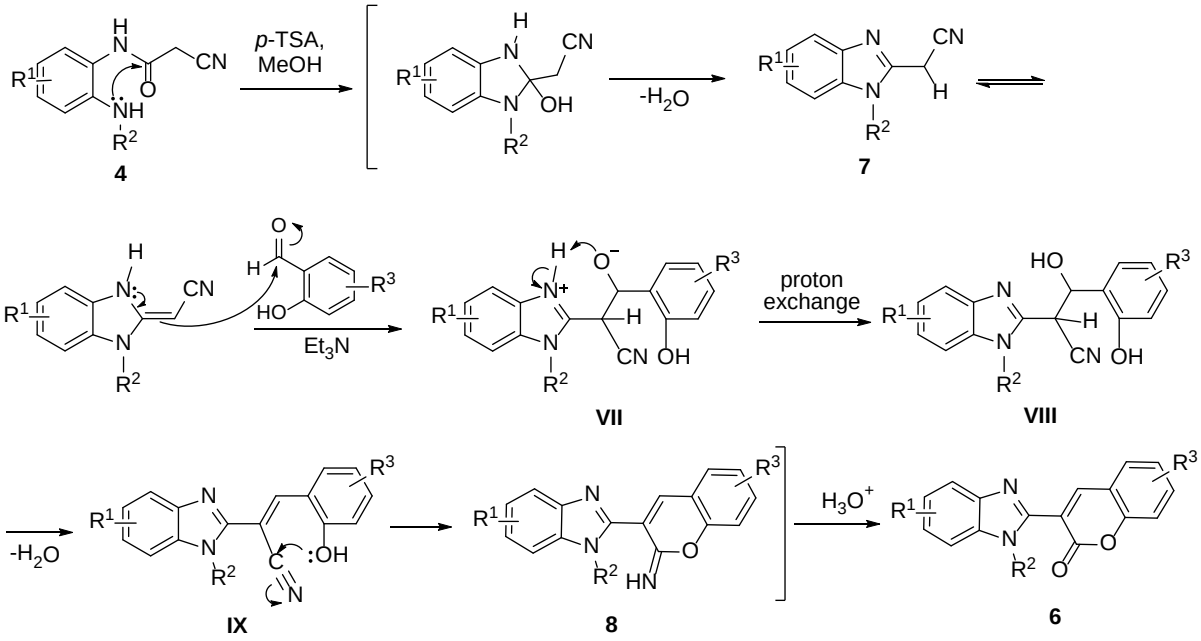


* Obtained from acetylation of **6**{*2,8,1*}

From the results obtained, a plausible mechanism was proposed for the conversion of **4** to **6** (Scheme 5). Initially, intramolecular dehydrative cyclization reaction of **4** in presence of *p*-TSA yielded the benzimidazole intermediate **7**. Nucleophilic attack of **7** on the carbonyl carbon of salicylaldehyde yielded **VII**, which subsequently converted to the intermediate **VIII** after intramolecular proton exchange. Subsequent dehydration of **VIII** yielded the nitrile intermediate **IX**. An intermediate 2-(2-imino-2*H*-chromen-3-yl)-1*H*-benzimidazole **8**, obtained by the intramolecular cyclization of **IX** was isolated and characterized by its mass, NMR (^{1}H and ^{13}C) and X-ray crystal structure data (Figure 3). Finally, acid-catalyzed hydrolysis of **8** furnished the

desired 2-coumarinylbenzimidazole **6**. It is worthy to mention that the lactone ring remains intact under acidic condition. The intermediate **7** was isolated and characterized by mass and NMR (^{1}H and ^{13}C) spectroscopy. (see spectroscopic data in the SI)

Scheme 5. A Plausible Mechanism for the Product (6) Formation



Coumarin linked benzimidaole derivatives were screened for their porcine kidney DAO (*pk*DAO) inhibitory effects. The cell-based DAO assay was performed at the 20.8 μM drug concentration and cell-base DAO assay was performed at 100 and 10 μM concentrations for all the compounds. The half maximal inhibitory concentration (IC_{50}) is a degree of the effectiveness of a substance in inhibiting a specific biological function. All the compounds were screened for their enzymatic activities. The synthetic compound **6**{*2,4,1*} was shown to possess the strongest *pk*DAO effect with IC_{50} ranging from 46.70 to 62.79 μM. (See the SI, Table 2 for complete assay)

CONCLUSION

In conclusion, we have developed an efficient and rapid combinatorial synthesis of novel coumarin linked benzimidazole heterocycles **6** using *p*-TSA, triethylamine and hydrochloric acid for the intramolecular cyclization, Knoevenagel condensation and imine hydrolysis, respectively through a one-pot, three-step sequential reactions. It was found that the scope of the one-pot

process is broad and this approach did avoid time-consuming and costly purification of each synthetic intermediates to obtain 2-(coumarin-3-yl)benzimidazoles in excellent yield. Among the synthesized molecules, the final target compound **6**{*2,4,1*} exhibited strongest porcine kidney DAO activity (IC_{50} range = 46.70 to 62.79 μM).

EXPERIMENTAL PROCEDURES

A Representative Procedure for One-pot, Three-step Synthesis of 2-(Coumarin-3-yl)benzimidazoles 6{*2,2,1*}. To a methanolic (20 mL) solution of **4**{*2,2*} (0.29 g, 1.0 mmol) in a 100 mL round bottom flask was added *p*-TSA (0.86 g, 5.0 mmol). The reaction mixture was heated at 50 ^{o}C for 4h. After cooling the reaction mixture to room temperature, salicylaldehyde (**5**, 0.18 g, 1.5 mmol) and triethylamine (1.01 g, 10 mmol) were added and the reaction mixture was stirred at 50 °C for 5h. Subsequently, HCl solution (10 mL, 1N) was added and the reaction mixture was continuously stirred for 2h at the same temperature until the reaction was completed (monitored by TLC). The organic solvent was removed by reduced pressure distillation and the residue obtained was washed with saturated sodium bicarbonate solution and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under vacuum to give the crude product. Further purification was achieved by column chromatography (EA:Hexane = 1:2) to give **6**{*2,2,1*} (0.32 g, 88 % yield).

Methyl 2-(coumarin-3-yl)-1-*N*-propyl-1*H*-benzimidazole-5-carboxylate 6{*2,2,1*}: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.32 (s, 1H), 8.06 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.66 – 7.61 (m, 2H), 7.46 (t, *J* = 8.4, 2H), 7.38 (t, *J* = 7.4, 1H), 4.24 – 4.20 (m, 2H), 3.95 (s, 3H), 1.85 (d, *J* = 7.4 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 158.9, 154.4, 149.7, 147.3, 142.6, 139.0, 133.3, 128.9, 125.1, 124.7, 124.7, 122.5, 119.3, 118.5, 116.8, 110.1, 52.1, 47.1, 22.9, 11.3. MS (ESI) m/z: 363.2 [M+H]; HRMS (ESI) m/z *calcd.* for $C_{21}H_{18}N_2O_4$: 363.1339 [M+H], found 363.1343.

ASSOCIATED CONTENTS

Supporting Information

Full characterization and spectroscopic data (^{1}H and ^{13}C NMR, LRMS and HRMS) of compounds **6** and experimental procedure for intermediates are included in the SI file.

CCDC 1506687 contains the supplementary crystallographic data for **8**{*2,3,1*}.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

DMSO	dimethylsulfoxide
EA	ethyl acetate
HOBt	1-hydroxybenzotriazole
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
MeOH	methanol
THF	tetrahydrofuran
p-TSA	*p*-toluene sulphonic acid
TEA	triethylamine
h	hour
eq	equivalent

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