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AN OPTIMIZED SYNTHETIC PROTOCOL FOR BIOLOGICALLY RELEVANT, HIGHLY FUNCTIONALIZED BENZOFURANS

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ABSTRACT: We report here the optimization of an oxidative Michael addition protocol for the synthesis of highly functionalized, biologically relevant benzofurans on gram scale. Rigorous screening of reaction conditions including solvent, atmosphere, base and incubation time, and development of a straight forward precipitation step afforded benzofurans in greater than 95% purity for direct use in biological assays.

INTRODUCTION: Functionalized benzofuran moiety represents an important building block and structural motif in many natural bioactive molecules with a wide range of activities such as antiatherosclerotic [1], antitumor [2, 3], antifungal [4], antipsychotic [5, 6], analgesic [7], estrogen receptor modulator [8, 9] and anti-osteoarthritic [10] agents. Recently, our group developed a group of small molecule regulators of thrombin based on a di-hydroxylated benzofuran scaffold [11, 12]. These regulators are the first molecules that allosterically inhibit thrombin and, thus form an important class of inhibitors with futuristic potential. Literature is replete with a good number of synthetic protocols available for preparing benzofurans.

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Most methodologies described in the literature for this purpose utilize either intra-molecular cyclization [13, 14], electrochemical [15], metathesis [16] or enzymatic annulations [17, 18], reactions. While generally useful, each of these suffer from one or more issues such as low yield, multiple steps, tedious isolation/purification procedure, or expensive raw materials. These issues introduce significant challenges, especially upon scale up, resulting in poor applicability for structure – activity studies. Additionally, these reactions are not particularly useful in synthesizing highly functionalized benzofuran scaffold, i.e., with substituents on at least 3 nuclear positions.

In 2006, Pei *et al.* reported a one-step oxidative procedure to synthesize a highly functionalized hydroxylated benzofuran from catechol using the oxidative-Michael addition reaction.[19] This protocol is attractive because of its one-pot nature and its potential in the generation of substituted benzofurans.

However, multiple attempts to exploit this reaction in the synthesis of our thrombin inhibitors showed that the reported reaction also suffers from low yield and difficulties of purification in the manner described above for other benzofuran scaffold synthesis. This severely limited the applicability of this rather simple and attractive reaction for structure–activity studies. Thus, we decided to study the influence of reaction conditions on the yield and ease of product isolation to assess its potential in drug discovery. We report that the one-pot oxidative Michael addition is an effective and simple approach for generating highly functionalized benzofurans. We develop a simple synthetic protocol that allows excellent scalability up to 10 g so as to enable studies in animal models.

EXPERIMENTAL: Reagents, chemicals and solvents were purchased either from Sigma-Aldrich (Milwaukee, WI) or Fisher (Pittsburgh, PA) and were used as received. Reagent solutions and chemicals were handled under inert nitrogen atmosphere using syringe techniques for liquid and dried spatula for solids. All the organic layers during work-up were dried using anhydrous sodium sulfate. ^{1}H and ^{13}C NMR spectra were recorded at either 300 or 400 MHz (Varian Mercury or Bruker Ultrashield^{TM} Plus) in appropriate deuterated solvents including $CDCl_3$ and Acetone-D_6. Signals are reported in ppm with the internal chloroform and acetone signals at 7.26 and 2.09, respectively, as standards. The data is reported as chemical shifts (ppm) and splitting pattern is described as (s = singlet, d = doublet, t = triplet, m = multiplet) with coupling constant(s) (Hz), and integration. Mass spectrometry was performed on all synthesized molecules using a Micromass ZMD4000 single quadrupole mass spectrometer or ACQUITY tandem quadrupole mass spectrometer with ESI ionization probe operating in positive ion mode (Waters Corp., Milford, MA).

General procedure of synthesis of Benzofurans: A reaction mixture of catechol (20 mmol) ethyl acetoacetate (20 mmol) and *N*, *N*-Diisopropylethylamine (20 mmol) was firstly stirred for 5 min. Ethanol (150 mL) and then water (50 mL) was added to the slurry. After stirring for 10 minutes, sodium iodate (10 mmol) was added. The reaction mixture was stirred for 10 hours at room temperature and quenched with 400 mL of ethyl acetate and washed with 150 mL of brine plus 30 mL of 0.5 N HCl. The organic layer was dried over sodium sulfate and concentrated down.

The solid residue obtained was dissolved in ethyl acetate (300 mL) and then the volume was reduced by half to produce a slurry. The precipitate was collected by centrifugation as the desired product with >95% purity in a yield of 40-70 %.

1: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.65 (s, 3H), 3.97 (s, 3H), 6.99 (s, 1H), 7.43 (s, 1H). ^{13}C NMR ($CDCl_3$, 300) δ 14.96, 60.54, 97.55, 106.92, 110.33, 119.06, 143.39, 145.92, 147.71, 161.34, 165.48. ESI MS (+ve) m/z calcd. for $C_{11}H_{10}O_5$ $[(M+H)^+]$ 223.05, found 223.0609.

2: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (t, *J* =24 Hz, 3H), 2.63 (s, 3H), 4.31 (q, *J* =24 Hz, 2H), 6.95 (s, 1H), 7.37 (s, 1H). ^{13}C NMR ($CDCl_3$, 300) δ 13.40 13.48 61.33 99.93 107.79 114.00 118.54 139.23 147.28 150.45 164.37 165.49. ESI MS (+ve) m/z calcd. for $C_{12}H_{12}O_5$ $[(M+H)^+]$ 237.06, found 237.0766.

3: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.60 (s, 9H), 2.63 (s, 3H), 6.96 (s, 1H), 7.39 (s, 1H). ^{13}C NMR ($CDCl_3$, 300) δ 14.29 28.48 80.89 94.12 105.86 110.19 119.27 143.14 144.90 147.56 162.11 163.88. ESI MS (+ve) m/z calcd. for $C_{14}H_{15}O_5$ $[(M+Na)^+]$ 287.08, found 287.0897.

4: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.63 (s, 3H), 3.41 (s, 3H), 3.72 (t, *J*=12 Hz, 2H), 4.46 (t, *J*=12 Hz, 2H), 6.96 (s, 1H), 7.39 (s, 1H). ^{13}C NMR ($CDCl_3$, 400) δ 14.48 55.77 58.96 70.67 103.45 103.46 108.87 119.65 143.41 147.95 149.17 162.71 164.49. ESI MS (+ve) m/z calcd. for $C_{13}H_{14}NaO_6$ $[(M+H)^+]$ 288.03, found 288.2907.

5: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.71 (s, 3H), 4.85 (d, 2H), 5.22 (dd, 1H), 5.42 (dd, 1H), 6.00 (m, 1H), 6.91 (s, 1H), 7.46 (s, 1H). ^{13}C NMR ($CDCl_3$, 400) δ 16.36 67.53 99.69 110.66 116.09 119.96 141.73 146.05 151.33 152.58 165.90 167.00. ESI MS (+ve) m/z calcd. for $C_{13}H_{12}O_5$ $[(M+H)^+]$ 249.07, found 249.0767.

6: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.03 (t, *J*=7.2 Hz, 3H), 1.82 (t, *J*=7.2 Hz, 2H), 2.70 (s, 3H), 4.28 (t, *J*=7.2 Hz, 2H), 7.00 (s, 1H), 7.47 (s, 1H).^{13}C NMR ($CDCl_3$, 400) δ 12.10 14.28 21.97 60.64 98.01

106.51 107.68 118.92 141.21 142.75 148.35 159.49 167.59. ESI MS (+ve) m/z calcd. for $C_{13}H_{14}O_5$ [(M+H) $^+$] 251.09, found 251.0920.

7: ^{1}H NMR (Acetone-d_6, 400 MHz) δ 1.25 (t, *J*=7.6 Hz, 3H), 1.39 (t, *J*=24 Hz, 3H), 3.10 (t, *J*=7.6 Hz, 2H), 4.31 (t, *J*=24 Hz, 2H), 6.97 (s, 1H), 7.39 (s, 1H). ^{13}C NMR (Acetone-d_6, 400) δ 12.55 14.66 22.16 60.60 98.40 107.16 116.18 120.76 141.24 143.87 144.90 145.99 167.12. ESI MS (+ve) m/z calcd. for $C_{13}H_{14}O_5$ [(M+H) $^+$] 251.09, found 251.0917.

8: ^{1}H NMR (Acetone-d_6, 400 MHz) δ 0.97 (t, *J*=7.6 Hz, 3H), 1.39 (t, *J*=24 Hz, 3H), 1.73 (m, 2H), 3.10 (t, *J*=7.6 Hz 2H), 4.33 (t, *J*=24 Hz, 2H), 6.97 (s, 1H), 7.40 (s, 1H). ^{13}C NMR (Acetone-d_6, 400) δ 13.76 14.29 21.42 30.27 60.54 97.64 106.50 108.35 118.90 141.17 142.77 148.38 158.56 166.51. ESI MS (+ve) m/z calcd. for $C_{14}H_{16}O_5$ [(M+H) $^+$] 265.01, found 264.1077.

9: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (t, *J*=24 Hz, 3H), 4.38 (t, *J*=24 Hz, 2H), 7.01 (s, 1H), 7.46 (m, 3H), 7.56 (s, 1H), 7.97 (m, 2H). ^{13}C NMR ($CDCl_3$, 400) δ 14.32 60.61 94.10 106.46 109.07 120.04 128.03 129.18 129.29 129.83 129.89 143.47 145.88 148.16 159.90 164.11. ESI MS (+ve) m/z calcd. for $C_{17}H_{14}O_5$ [(M+H) $^+$] 299.09, found 299.0923.

DISCUSSION: Our first attempt at oxidative Michael addition of ethyl acetoacetate onto catechol using the method reported by Pei *et al* [19] (6 mmol $NaIO_3$, 5 mmol catechol, 5 mmol ethyl acetoacetate, 5 mmol pyridine and 50 mL EtOH:H_2O [9:1] solvent) gave the desired product in yields below 20%. In addition, several rounds of chromatographic purification were needed to gain a purity of >95%. To optimize the reaction conditions, we began with a study of the influence of solvent, base and reaction time.

Solvent composition played a crucial role in defining the reactivity of components. Detailed studies indicated that the reaction did not proceed at all in organic solvents, anhydrous acetonitrile, anhydrous ethanol or mixtures of the two (**Table 1**). In contrast, ethanol–water mixture allowed oxidative Michael's product formation. In fact, the proportion of water in the mixture was critical. Whereas, a 50:50 or 96:4 (v/v) ethanol–water mixture was completely ineffective, a 75:25 mixture was found to be optimal. Although the exact reason for this stringency is difficult to decipher, solubility of the raw materials and benzofuran.

To assess the dependence of the yield on the type of base used to drive the reaction, several bases were screened (**Table 2**). As the pK_a of the base increases, the reaction yield improved consistently with diisopropylethyl-amine (DIPEA) resulting in a 50% yield. This is reasonable because better deprotonation of acidic hydrogen from ethyl actetoacetate can be expected to facilitate coupling.

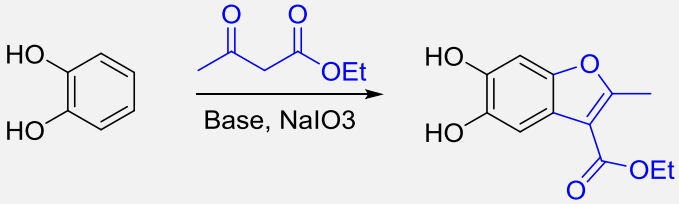
Yet, stronger bases, such as *t*-butoxide (pK_a=19), failed miserably. Using DIPEA to screen for best reaction time suggested that 10-12 h of incubation was most optimal (Table 2). In fact, longer reaction time led to a significant decrease in yield, which we reasoned as arising from oxidative degradation of the benzofuran product.

The development of higher yielding synthetic protocol enabled a precipitation step that readily gave highly pure benzufuran product, which can be used directly in initial biological assays. In fact, quick purification of the benzofuran product through 1) direct precipitation in ethyl acetate and 2) fast liquid chromatography gave purities in excess of 97% resulting in a highly effective procedure for the synthesis of highly functionalized benzofuran derivatives. This led to a simple synthetic protocol in which catechol (20 mmol), ketoester (20 mmol) and DIPEA (20 mmol) were mixed first to form a slurry, then ethanol–water mixture (3:1) and sodium iodate (10 mmol) added in that order. The reaction was allowed to proceed at room temperature for 10 h. The benzofuran product could be obtained in 95% purity by direct precipitation from ethyl acetate.

To assess the feasibility of employing this reaction to generate biologically relevant, highly functionalized benzofurans, various commercially available alkyl acetoacetates including methyl, ethyl, propyl, *t*-butyl, phenyl, methoxy ethyl and allyl ester were investigated. In nearly all cases, the optimized synthetic protocol developed here resulted in moderate to good yields (40–70%) on 10 g scale, which enabled our drug discovery efforts. It was observed that the yield improved with polarity of the esters, such as methoxyethyl

ester, while it decreased with the hydrophobicity of esters, such as *t*-butyl and phenyl ester (**Table 3**). However, the scaffolds lacking the catechol moiety such as o-phenylene diamine, 2-amino phenol, dihydroxyl naphthalene and guaiacol did not resulted in any product formation. Interestingly, our simple purification method of direct precipitation in ethyl acetate was found to apply equally well with this series of esters.

TABLE 1: THE EFFECT OF SOLVENT COMPOSITION [a]



Solvent	Yield[b]
EtOH	0
CH_3CN	0
CH_3CN : EtOH (80:20)	0
DMF	0
H_2O	0
H_2O : EtOH (96:4)	0
H_2O : EtOH (90:10)	7%
H_2O : EtOH (80:20)	18%
H_2O : EtOH (75:25)	25%
H_2O : EtOH (60:40)	15%
H_2O : EtOH (50:50)	0
H_2O : EtOH (75:25)	0 (heating at 50 °C)
H_2O : EtOH (75:25)	0 (under nitrogen)

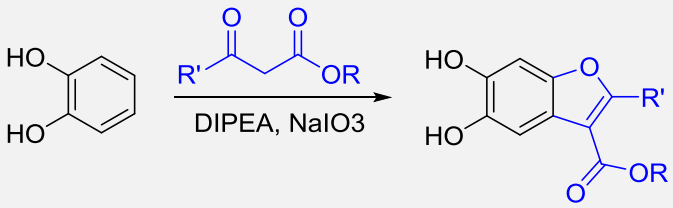
[a]The reaction of catechol (20 mmol) with ethyl acetoacetate (20 mmol) in the presence of pyridine (20 mmol) and $NaIO_3$ (10 mmol) at RT for 14 h was studied. [b]Represents isolated yield. R and R'represent ethyl and methyl group respectively.

TABLE 2: THE EFFECT OF BASE AND TIME ON THE OXIDATIVE MICHAEL ADDITION REACTION [a]

	% Yield[b]
Influence of Base	
Pyridine (1 eqiv.)	25
Pyridine (2 eqiv.)	10
Et_3N (1 eqiv.)	40
DIPEA (1 eqiv.)	50
t-BuOK (1 eqiv.)	0
Influence of Reaction Time [c]	
8 h	15
10 h	60
12 h	50
14 h	40
24 h	20

[a]The reaction of catechol (20 mmol) with ethyl acetoacetate (20 mmol) in the presence of 20 mmol base and 10 mmol $NaIO_3$ at RT for 12 h was studied. [b]Represents isolated yield. [c]DIPEA (20 mmol) was used as the base.

TABLE 3: THE EFFECT OF VARIATION IN THE STRUCTURE OF STARTING MATERIALS [a]



	-R'	-R	% Yield[b]
1	$-CH_3$	$-CH_3$	60
2	$-CH_3$	$-CH_2CH_3$	60
3	$-CH_3$	$-C(CH_3)_3$	40
4	$-CH_3$	$-CH_2CH_2OCH_3$	70
5	$-CH_3$	$-CH_2CH=CH_2$	60
6	$-CH_3$	$-CH_2CH_2CH_3$	45
7	$-CH_2CH_3$	$-CH_2CH_3$	40
8	$-CH_2CH_2CH_3$	$-CH_2CH_3$	40
9	$-C_6H_5$	$-CH_2CH_3$	40

[a]The reaction of catechol or catechol derivative (20 mmol) with dicarbonyl compound (20 mmol) was studied in the presence of DIPEA (20 mmol) and $NaIO_3$ (10 mmol) in H_2O:EtOH mixture (1:3) at RT for 10 h. [b]Represents isolated yields.

CONCLUSION & SUMMARY: In summary, we report here an optimized oxidative Michael addition protocol for the synthesis of highly functionalized benzofurans. The protocol was developed through rigorous screening of reaction conditions including solvent, atmosphere, base and reaction time. Optimization of conditions led a straight forward precipitation step that afforded benzofurans in greater than 95% purity for direct use in biological assays. These benzofurans synthesized herein can be readily converted to other biologically relevant molecules, such as the highly sulfated aromatic molecules developed earlier [11, 12]. The overall advantages of this protocol include relative low cost of reagents, short time and ease of purification to produce gram scale synthesis of highly hydroxylated benzofurans, which has been found difficult to achieve.

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