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Synthesis of Isoflavones by Tandem Demethylation and Ring-Opening/Cyclization of Methoxybenzoylbenzofurans

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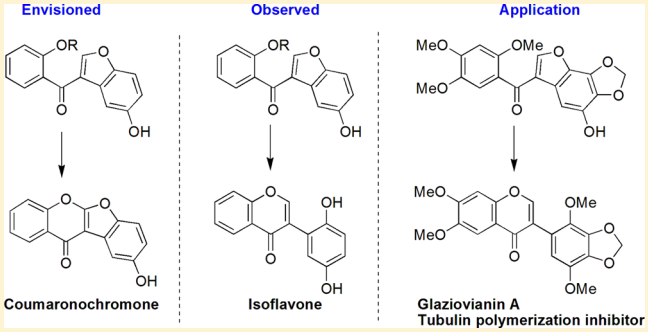
Supporting Information

ABSTRACT: The unexpected conversion of benzoylbenzofurans into isoflavones through an intramolecular cascade that involves deprotection and ring-opening/cyclization is described. This was discovered in an investigation of the possible transformation of benzoylbenzofurans into coumaronochromones. This route affords isoflavones in two major steps from acetophenones and benzoquinones. The transformation was validated by synthesizing differently substituted isoflavone derivatives and further applied to a concise synthesis of a potential anticancer lead compound, glaziovianin A (**1**).



Flavonoids are phenolic secondary metabolites abundantly available in fruits and vegetables.[1,2] In the plant kingdom, these natural compounds are, among others, responsible for protection of the plants against predatory insects and microbes and also for the colorful (blue, red, purple) pigments needed to attract pollinating insects.[3] Flavonoids are characterized by their two aromatic rings linked through a three-carbon bridge, forming a $C_6-C_3-C_6$ carbon skeleton that is derived from the acetate/malonate and shikimic acid biosynthetic pathways. They are divided into three main classes, namely, flavonoids (2-phenylchromen-4-ones), isoflavonoids (3-phenylchromen-4-ones), and neoflavonoids (4-phenylcoumarins). Structural modifications may include hydroxylation, methylation, prenylation, glycosylation, and varying the oxidation state on the C_3 unit, resulting in several analogues.[2,4,5] Of the three flavonoid classes, isoflavonoids offer considerable diversity and are further grouped into subclasses that include coumaronochromones, rotenoids, pterocarpans, 3-arylcoumarins, and coumestans.[6] The different isoflavonoid compounds exhibit a myriad of biological activities such as estrogenic, antifungal, antibacterial, cytotoxicity against cancer cell lines, immunosuppressive, anti-HIV, and antiplatelet activities.[1,6−11] Some of the biologically active natural isoflavonoids are glaziovianin A (**1**),[7] hirtellanine A (**2**),[8] euchretin F (**3**), formosanatin C (**4**),[9,10] erybreadin (**5**), dabinol (**6**), and nitidulin (**7**) (Figure 1).[11] The striking feature in these bioactive isoflavonoids is the presence of the 2′,5′-oxygenation pattern, as well as the prenyl group on either the A- or B-ring. As a result of their interesting structures and important biological activities, the synthesis of isoflavonoids has attracted much interest.[12−15]

One of the long-established and still commonly used methods to access the isoflavone scaffold is through the deoxybenzoin route, which proceeds via Friedel−Crafts acylation of a phenol with phenylacetic acid followed by acid-mediated ring closure of the resulting *o*-phenol.[16−18] This route, however, suffers from the rather limited access to diverse derivatives of phenylacetic acid. Other major synthetic routes include oxidative rearrangement of chalcones,[19−23] ring-closing metathesis with Grubbs' catalyst,[24] Suzuki coupling of 3-halochromones with aryl boronic acids,[25,26] Negishi cross-coupling reaction of 3-halochromones with arylzinc halides or 3-zincated chromones with aryl halides,[27,28] the Wacker−Cook tandem conversion of α-methylene deoxybenzoins,[29] and the Cu(I)-mediated cyclization of 3-(2-bromophenyl)-3-oxopropanal.[30] Despite the numerous synthetic methods, several significant drawbacks remain. Some of the methods utilize hazardous materials and harsh reaction conditions that are intolerant to some functional groups found in naturally occurring products. Other methods such as the Suzuki, Negishi, and Grubbs ring-closing metathesis utilize expensive transition metals and starting building blocks that are synthesized in several steps, which often lead to lower yields of the final products.[24,31]

Our aim was to investigate alternative synthetic strategies for the synthesis of isoflavonoids, with an initial focus on the preparation of coumaronochromones. Currently, coumaronochromones are synthetically accessed from the 2′-hydroxyisoflavones via oxidative cyclization involving the 2′-hydroxy group and C-2. We envisioned to prepare this tetracycle from the 3-(2′-hydroxybenzoyl)benzo[*b*]furan precursors, through late-stage construction of the chromone core by cyclization involving the HO-2′ and the C-2 benzoyl groups, as illustrated in Scheme 1. In an attempt to prepare the requisite 3-(2′-

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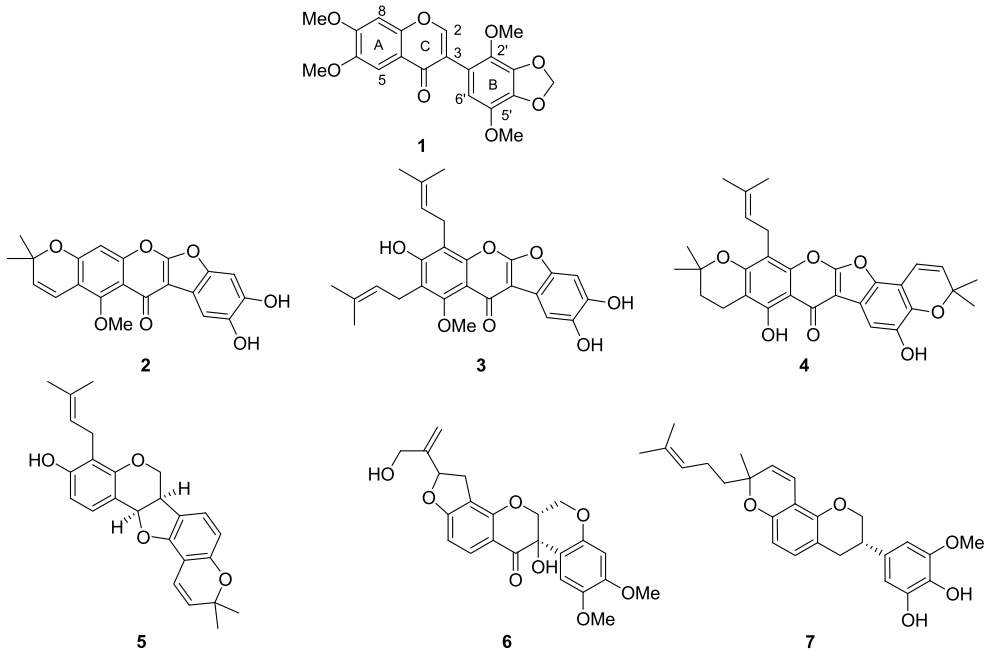
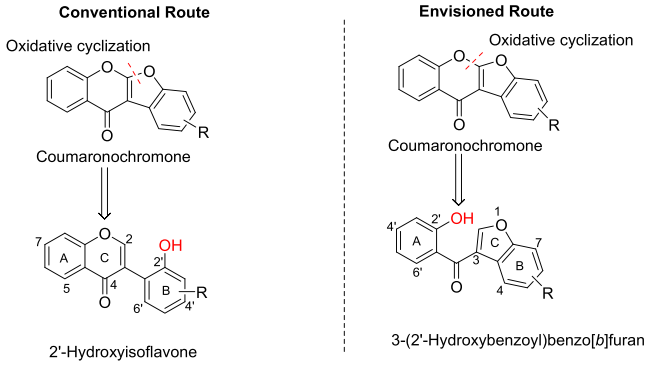


Figure 1. Examples of biologically active isoflavonoids.

Scheme 1. Conventional and Envisioned Route for Coumaronochromones



hydroxybenzoyl)benzo[*b*]furan intermediates, we unexpectedly obtained the 2′-hydroxyisoflavones. Although our initial objective was to investigate the possible conversion of benzoylbenzofurans into coumaronochromones, the formation of the 2′-hydroxyisoflavones via the benzoylbenzofuran intermediates provides an alternative route for the synthesis of isoflavones and other isoflavonoids. The 2′-hydroxyisoflavones could serve as precursors to the synthesis of complex isoflavonoids.

In order to ascertain the utility of this protocol, we probed the functional group tolerance and applicability of the method on the synthesis of differently substituted isoflavones, including the natural compound glaziovianin A (**1**). Glaziovianin A (**1**) was isolated from *Ateleia glazioviana* Baillon (Leguminosae), and it was found to exhibit cytotoxicity against a panel of cancer cell lines and to inhibit tubulin polymerization.[7] Glaziovianin A (**1**) and its analogues have been synthesized in order to study their structure−activity relationship and mechanisms of action.[32] The reported syntheses were based on the Suzuki−Miyaura coupling reaction and Cu(I)-mediated cyclization of *β*-ketoaldehydes.[32−34] Therefore, the synthetic method reported in this study provides an alternative approach to the synthesis of naturally occurring isoflavones and analogues. Furthermore, the current approach uses readily available commercial reagents and simple experimental protocols and provides access to diverse polyhydroxylated and alkoxy-substituted isoflavones in two major steps. The synthesis of isoflavones from benzoylbenzofurans has not been reported before.

RESULTS AND DISCUSSION

As previously mentioned, the initial plan was to investigate the conversion of the 3-(2′-hydroxybenzoyl)benzo[*b*]furans into coumaronochromones. In order to test this hypothesis, the requisite benzoylbenzofuran precursors had to be synthesized. There are several routes through which benzoylbenzofurans can be synthesized; however, most of these have been employed for the preparation of the 2-substituted benzoylbenzofurans. The exception is the modified Larock method applied by Flynn and co-workers.[35] Another method that has been used to synthesize benzoylbenzofurans devoid of substituents at C-2 is the conjugate addition of enaminoketones to 1,4-benzoquinones. The latter method was employed in this study because it utilizes readily available starting acetophenones and benzoquinones and also affords the 3-benzoylbenzofurans in a few steps and in good yields.[36−38] As shown in Scheme 2, the synthesis was initiated by reacting the TBDMS-protected acetophenone **8** with *N*,*N*-dimethylformamide dimethylacetal (DMF−DMA) followed by benzoquinone in HOAc. This gave the benzoylbenzofuran **9** in a low yield and the chromone **11** in a high yield.[39] An attempt to convert the anticipated benzoylbenzofuran **9** into coumaronochromone **10** by oxidative cyclization rather yielded isoflavonequinone **13**, which upon reduction with $SnCl_2$ gave the dihydroxyisoflavone **12**. Analysis of the NMR data of the isoflavone **12** and comparison of the spectroscopic data to those of the presumed benzoylbenzofuran **9** surprisingly showed that the NMR data sets for the two compounds were identical, thereby suggesting that the benzoylbenzofuran **9** was not isolated initially, but the isoflavone **12**. The ^{1}H

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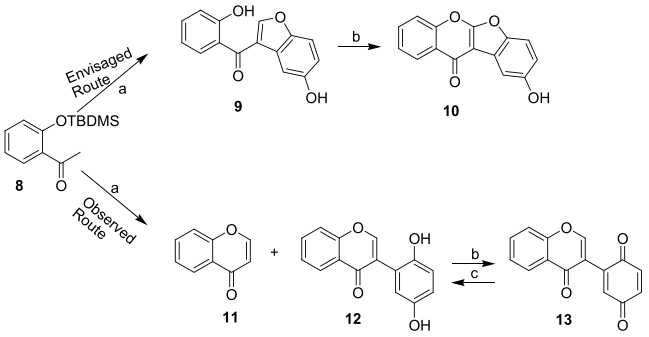
Scheme 2. Unexpected Formation of Isoflavone[a]



[a]Conditions: (a) i. DMF−DMA, ii. benzoquinone, HOAc; (b) DDQ, CH_2Cl_2; (c) $SnCl_2$, CH_2Cl_2.

NMR spectrum of the isoflavone **12** displayed three one-proton singlets, consisting of a sharp singlet resonating at δ_H 8.35 (H-2) and two broad singlets resonating at δ_H 8.65 and 8.79 for HO-2′ and HO-5′. Signals for seven aromatic protons appearing as an ABX spin system at δ_H 6.61−6.65 (m, overlap, H-4′ and -6′) and δ_H 6.71 (d, J = 8.6 Hz, H-3′) for the B-ring protons and an AMPX spin system at δ_H 7.50−7.54 (m, H-6); 7.68 (dd, J = 8.5, 0.6 Hz, H-8); 7.81−7.86 (m, 1H, H-7); 8.11 (dd, J = 8.5, 1.5, Hz, H-5) for the A-ring aromatic protons were displayed. It is noteworthy that the NMR spectra of both the benzoylbenzofuran **9** and isoflavone **12** would have the same number of signals because they possess similar aromatic systems and the oxymethine; thus it would be difficult to distinguish between the two on the basis of ^{1}H NMR spectra alone. The ^{13}C NMR spectrum of isoflavone **12** showed the presence of 15 carbon signals, with the carbonyl carbon resonating at δ_C 175.3, which is indicative of conjugation for the cyclized system.

The structure of compound **12** was further confirmed by the HMBC correlations (Figure 2). The hydroxy proton at C-2′

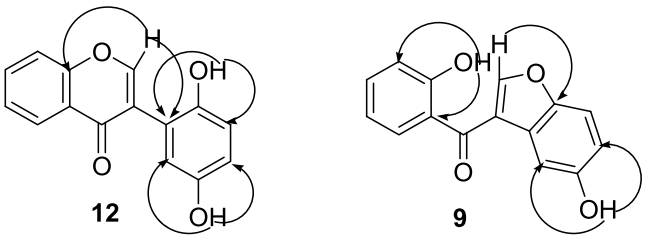
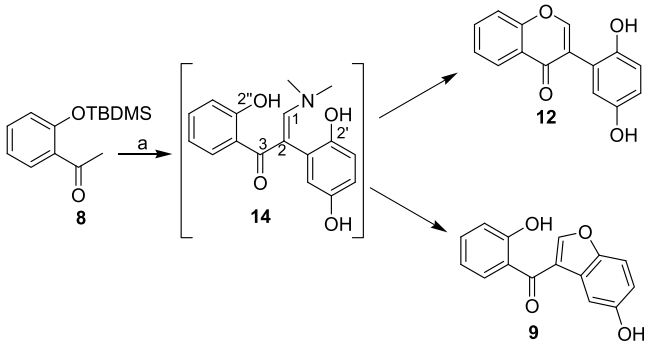


Figure 2. Key HMBC correlations for 2′,5′-dihydroxyisoflavone (**12**) and 2′,5-dihydroxybenzoylbenzofuran (**9**).

(δ_H 8.65, HO-2′) correlated to C-1′ and C-3′, and the hydroxy proton at C-5′ (δ_H 8.79, HO-5′) correlated to C-4′ and C-6′, which is indicative of the presence of a hydroquinone B-ring of the isoflavonoid. This was confirmed by the HMBC correlations of H-2 (δ_H 8.35) to C-1′ and to the oxygen-linked tertiary carbon C-8a (δ_C155.7). In contrast to the isoflavone, the hydrogen-bonded HO-2′ proton (δ_H 10.53) of the benzoylbenzofuran **9** displayed correlations to C-1′ and C-3′ of the benzoyl group, while the HO-5 proton (δ_H 9.50) of the furan moiety correlated to C-4 and C-6, and H-2 (δ_H 8.46) to C-7a (δ_C 149.0). The synthesis of compound **9** is described later in Scheme 4.

It was therefore proposed that the nucleophilic addition of an enaminoketone (formed by condensation of acetophenone

Scheme 3. Proposed Intermediate for Formation of Isoflavone 12 or Benzofuran 9[a]



[a]Conditions: (a) i. DMF−DMA, 120 °C, ii. benzoquinone, HOAc, rt.

8 with DMF−DMA) to 1,4-benzoquinone proceeded via the formation of *tert*-butyl(dimethyl)silyl (TBDMS)-deprotected intermediate **14**, as shown in Scheme 3. Thus, with free hydroxy groups at C-2′ and C-2″, the enaminone intermediate **14** could undergo intramolecular cyclization to give either the benzoylbenzofuran **9** or the isoflavone **12**.

In order to prevent the premature cycloaddition via the 2″-hydroxy group, a more robust protecting group was used and the 3-benzoylbenzofurans were synthesized from 2-methoxyacetophenones. Thus, the condensation of acetophenone **15** with DMF−DMA and subsequent treatment of the resulting enaminoketone with 1,4-benzoquinone in HOAc gave the expected methoxybenzoylbenzofuran **16** in 86% yield. An attempt to demethylate **16** with BBr_3 at −78 °C unexpectedly yielded both the 3-(2′-hydroxybenzoyl)benzo[*b*]furan **9** in 18% yield and the isoflavone **12** in 11% yield, with more than half of the starting material recovered (Scheme 4).

Intrigued by this formation of an isoflavone from a benzoylbenzofuran, different conditions for demethylation were explored and led to the outcomes collated in Table 1. The demethylation reaction was repeated varying the temperature, the molar equivalents of BBr_3, and other demethylating agents. Allowing the temperature to increase from −78 to 0 °C resulted in a slight increase of the cyclized product (isoflavone), yet a significant amount of the starting material was recovered (entry 2). Interestingly, performing the reaction at temperature of 0 °C to rt resulted in complete deprotection−cyclization, converting all the starting material to isoflavone **12** (78%) (entry 3). The slightly lower than expected yields were attributed to possible decomposition due to higher temperatures. Next, a milder Lewis acid, $AlCl_3$, was used in an attempt to minimize the presumed decomposition. This, however, afforded the benzoylbenzofuran **9** exclusively in excellent yields (entry 4), and not a scintilla of the cyclized product was detected. The use of EtSH and $AlCl_3$ (entry 5) resulted in a complex mixture of products that were not characterized. Disappointingly, the reaction with 1 M trimethylsilyl iodide (TMSI) did not proceed at all (entry 6) and left the starting material intact. However, the more concentrated (98%) TMSI afforded the isoflavone in 68% yield and the benzofuran in trace quantities (4%). Owing to the instability of TMSI, we thought generating it in situ would yield better results; however, this led to a drop in the isoflavone yield (56%), as further increments of NaI and TMSCl resulted in the formation of more NaCl, making it harder to effectively stir the reaction mixture.

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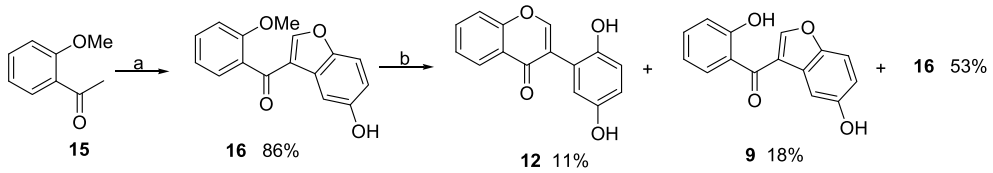
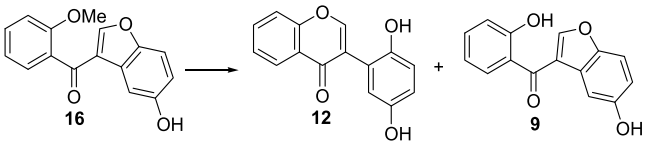
Scheme 4. Demethylation of Benzofuran 16[a]



[a]Conditions: (a) i. DMF−DMA, ii. benzoquinone, HOAc; (b) BBr_3, CH_2Cl_2, −78 °C.

Table 1. Screening of Demethylating Reagents



						yield (%)[a]	
entry	T (°C)	time (h)	solvent	demethylating reagent	mol equiv	12	9
1	−78	6	CH_2Cl_2	BBr_3	3	11	18
2	−78−0	3	CH_2Cl_2	BBr_3	3	28	33
3	0−rt	4	CH_2Cl_2	BBr_3	3	78	
4	0−rt	18	CH_2Cl_2	$AlCl_3$	6		91
5	0−rt	12	CH_2Cl_2	$AlCl_3$/EtSH	3	[b]	[b]
6	80	48	DMF	TMSI[c]	8	[d]	[d]
7	80	48	DMF	TMSI[e]	6	68	4
8	80	48	DMF	NaI/TMSCl	6	56	9

[a]Isolated yields. [b]Uncharacterized products. [c]1 M TMSI was used. [d]No reaction. [e]98% TMSI was used.

The best reaction conditions for the isoflavone formation were next employed to explore the substrate scope of this deprotection−cyclization using a panel of 3-benzoyl-5-hydroxybenzofurans (**17**−**39**) as shown in Table 2. In order to investigate the electronic effect of acetophenone substituents on demethylation−cyclization, various A-ring-substituted benzoylbenzofurans were tested. Treatment of the halo-substituted benzoylbenzofurans **17**−**20** with BBr_3 at 0 °C to rt rendered the 6-bromoisoflavone **40**, the 7-fluoroisoflavone **42**, and the 6-chloroisoflavone **44** in yields of 62%, 65%, and 60%, respectively (Table 2, entries 2−4). The corresponding halogenated benzoylbenzofurans **41**, **43**, and **45** were isolated in low yields. Outlying results were observed upon deprotection of 3-(5′-fluoro-2′-methoxybenzoyl)-5-hydroxybenzo[*b*]furan (**20**), which gave the isoflavone **46** in a low yield of 19% and the corresponding demethylated benzofuran **47** in 36% yield (entry 5). The low yields for the 6-fluoroisoflavone **46** could be due to the strong inductive electron-withdrawing effect of the fluorine atom, resulting in decreased nucleophilicity of the phenoxide intermediate during demethylation.

Since most naturally occurring isoflavones possess oxygenated substituents, the functional group tolerance of the developed synthetic route was next assessed on the benzoylbenzofuran precursors carrying oxygenated substituents. These included the dimethoxybenzoylbenzofurans **21** and **22** and the trimethoxybenzoylbenzofurans **23**, **24**, and **25** (entries 6−10). The benzoylbenzofuran **21** was treated with six molar equivalents of BBr_3 under the established conditions. The reaction afforded the methoxyisoflavone **48** and the trihydroxyisoflavone **49** in 43% and 23% yields, respectively (entry 6). Subjecting the 5-hydroxy-3-(2′,4′-dimethoxybenzoyl)benzo[*b*]furan (**22**) to the same conditions afforded the targeted isoflavone **50** in 6% yield and the demethylated benzoylbenzofuran **51** in 58% yield. The failure to obtain the isoflavone **50** in good yields under established demethylation conditions prompted the use of eight molar equivalents of BBr_3 and a reaction time of 6 h. This afforded the isoflavone **50** in slightly improved yield (27%), with the mono-demethylated benzoylbenzofuran **51** isolated in 30% yield (entry 7). Next, the effect of additional methoxy groups was evaluated using trimethoxy-substituted benzoylbenzofurans **23**, **24**, and **25**. The deprotection of 5-hydroxy-3-(2′,3′,4′-trimethoxybenzoyl)-benzo[*b*]furan (**23**) following the general procedure afforded the two isoflavones **52** and **53** in 2% and 21% yields, respectively and the mono-demethylated benzoylbenzofuran **54** in 47% yield (entry 8). Similarly, demethylation of the 5-hydroxy-3-(2′,4′,6′-trimethoxybenzoyl)benzo[*b*]furan (**24**) afforded the mono-demethylated benzoylbenzofuran **58** in higher yield (37%) than the targeted isoflavones **55** (24%), **56** (9%), and **57** (1%) (entry 9). Owing to the many products isolated from the demethylation of the trimethoxy-substituted benzoylbenzofurans, we attempted to circumvent this problem by aiming for a fully demethylated product and thus increased the reaction time to 6 h and molar equivalents of BBr_3 to 4 equiv per methoxy group for the third trimethoxy substrate **25**. Thus, the reaction of the 5-hydroxy-3-(2′,4′,5′-trimethoxybenzoyl)benzo[*b*]furan (**25**) with excess BBr_3 exclusively afforded the tetrahydroxyisoflavone **59** in 29% yield (entry 6). Although **59** was the sole product, the low yield of 29% could be attributed to decomposition due to the large excess of BBr_3. Based on the above observations, it can be postulated that the major limitation with the deprotection−cyclization of the benzoylbenzofurans bearing multiple methoxy groups is poor selectivity of the demethylation reaction, which leads to the formation of multiple-demethylated (mono, di, and tri)hydroxyisoflavones. Additionally, to achieve full deprotection, an excess of BBr_3 is required, which leads to decomposition and low yields of the targeted isoflavones.

With functional group tolerance of the A-ring substituents evaluated, the next focus involved the effect of the B-ring substituents on the deprotection−cyclization of benzoylbenzofurans to isoflavones. The reaction with halogen substituents on the B-ring (**26**−**29**) proceeded well, affording the isoflavone derivatives **60**, **61**, **63**, and **65** in yields ranging from 56% to 66% and the counterpart 2′-hydroxybenzoylbenzofurans in low yields (entries 11−14). However, with the electron-donating methyl groups on the B-ring, isoflavonequinones were more predominant and the hydroxyisoflavones were isolated in trace quantities (entries 15−22). The formation of isoflavonequinones could be attributed to the high susceptibility of electron-rich hydroquinones to oxidation. Autoxidation of hydroquinones to the corresponding benzoquinones is a known phenomenon and is reported to proceed

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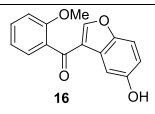
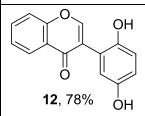
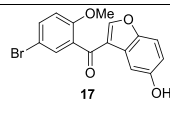
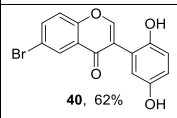
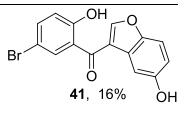
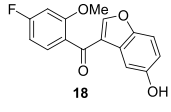
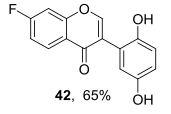
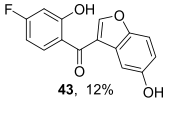
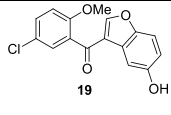
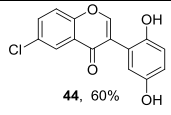
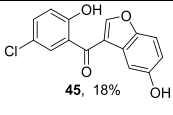
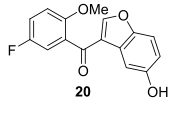
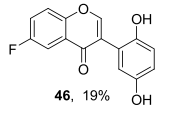
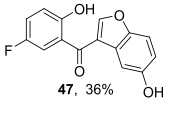
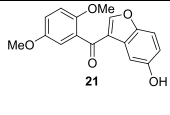
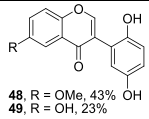
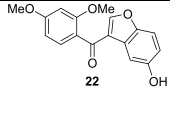
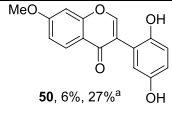
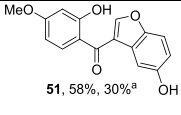
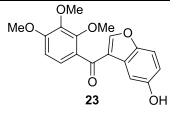
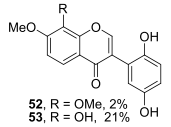
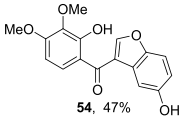
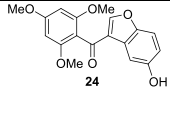
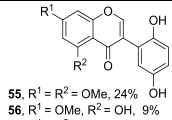
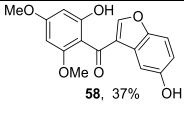
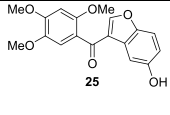
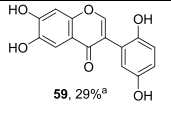
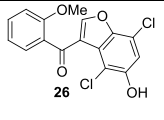
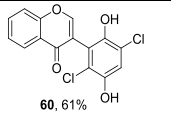
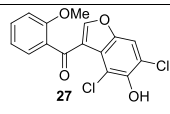
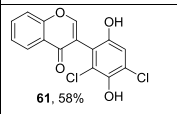
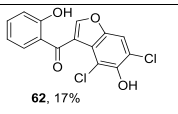
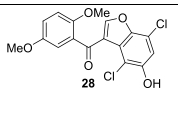
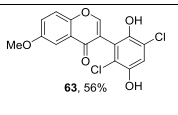
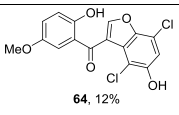
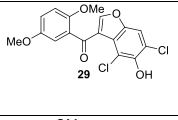
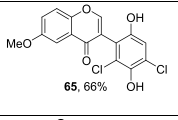
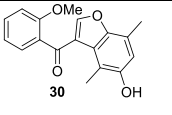
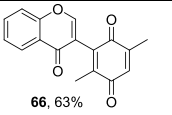
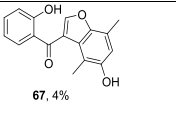
Table 2. Demethylation–Cyclization Scope

Entry	Benzoylbenzofuran Precursor	Deprotection Products	
		Isoflavone	Benzoylbenzofuran
1	 **16**	 **12**, 78%	
2	 **17**	 **40**, 62%	 **41**, 16%
3	 **18**	 **42**, 65%	 **43**, 12%
4	 **19**	 **44**, 60%	 **45**, 18%
5	 **20**	 **46**, 19%	 **47**, 36%
6	 **21**	 **48**, R = OMe, 43% **49**, R = OH, 23%	
7	 **22**	 **50**, 6%, 27%[a]	 **51**, 58%, 30%[a]
8	 **23**	 **52**, R = OMe, 2% **53**, R = OH, 21%	 **54**, 47%
9	 **24**	 **55**, $R^1 = R^2$ = OMe, 24% **56**, R^1 = OMe, R^2 = OH, 9% **57**, $R^1 = R^2$ = OH, 1%	 **58**, 37%
10	 **25**	 **59**, 29%[a]	
11	 **26**	 **60**, 61%	
12	 **27**	 **61**, 58%	 **62**, 17%

Entry	Benzoylbenzofuran Precursor	Deprotection Products	
		Isoflavone	Benzoylbenzofuran
13	 **28**	 **63**, 56%	 **64**, 12%
14	 **29**	 **65**, 66%	
15	 **30**	 **66**, 63%	 **67**, 4%
16	**31**	**68**, 57%	
17	**32**	**69**, 66%	
18	**33**	**70**, 42% **71**, 3%	
19	**34**	**72**, 11%	**73**, 55%
20	**35**		**74**, 64%
21	**36**	**75**, 18%	**76**, 49%
22	**37**	**77**, 68%	
23	**38**	**78**, 36%	**79**, 12%
24	**39**	**80**, 41%	

[a]Four equivalents of BBr_3 per methoxy group used; reaction time was 6 h.

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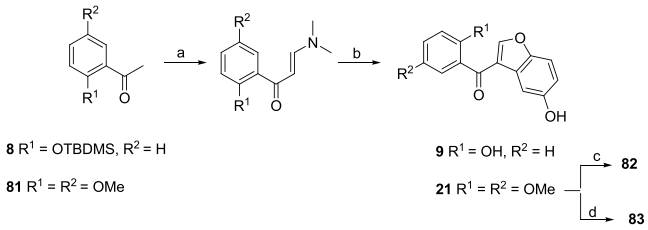
by a radical process involving initial formation of a *p*-semiquinone radical.[40−43] Despite the formation of the isoflavonequinones, the effect of the methyl substituents on the cyclization process and yields of the isoflavones/quinones is minimal and depends mostly on the positions of the methyl groups on the B-ring. Better isoflavone yields were obtained for a *p*-dimethyl arrangement as observed for compounds **66** (63%), **69** (66%), and **77** (68%), while slightly lower yields were obtained for the *m*-dimethyl analogues as seen for compounds **68** (57%) and **70** (42%). Contrarily, demethylation of compounds **34** and **36** resulted in exceptionally low yields of isoflavonequinones **72** and **75**, which were isolated in 11% and 18% yields, respectively. The major products were the corresponding 2′-hydroxybenzoylbenzofurans **73** (55%) and **76** (49%). Demethylation of the resorcinolic benzoylbenzofuran **35** with an *m*-dimethyl group failed to cyclize to a corresponding isoflavone/quinone; instead, the benzoylbenzofuran **74** was isolated in a 64% yield. The last set of benzoylbenzofurans to be tested were naphthobenzoylbenzofurans. The deprotection of naphthobenzoylbenzofurans **38** and **39** also afforded the corresponding isoflavonequinones **78** and **80** in 36% and 41% yield, respectively, with the deprotected benzoylbenzofuran **79** being isolated in a 12% yield.

Based on these findings, it is observed that the effect of the A-ring substituents on the deprotection−cyclization of benzoylbenzofurans to the corresponding isoflavones is influenced by mesomeric effects. Thus, these observations are in accordance with the literature regarding the reactivity of substituted phenoxides.[44−47] For the methoxy-substituted benzoylbenzofurans, good yields were obtained from the 2′,5′-dimethoxy-substituted benzoylbenzofurans, while the benzoylbenzofurans with resorcinol and pyrogallol moieties gave poor yields of the isoflavones, and the corresponding 2′-hydroxybenzoylbenzofurans were isolated in significant yields. The good yields of isoflavones obtained from the *p*-dimethoxybenzoylbenzofurans are attributable to the enhanced nucleophilicity of the *p*-methoxyphenoxide ions and subsequent ease of cyclization. In addition to the electronic effects, it was noted that the lower the BBr_3 equivalents, the better the isoflavone yields, as observed for the demethylation of benzoylbenzofurans containing one methoxy substituent, where a maximum of three molar equivalents of BBr_3 were used, in sharp contrast to the dimethoxy- and trimethoxy-substituted precursors, which required at least six molar equivalents of BBr_3. On the other hand, it was noted that the B-ring substituents did not have a significant impact on the ring-opening/cyclization process. However, when the B-ring was substituted with electron-donating groups, autoxidation was prevalent, resulting in the formation of isoflavonequinones.

In general, the demethylation of the 3-(2′-methoxybenzoyl)-benzo[*b*]furans resulted in either of three products, i.e., the 3-(2′-hydroxybenzoyl)benzo[*b*]furans, the 2′,5′-dihydroxy-isoflavones, and the isoflavone-2′,5′-quinones depending on the substituents and substitution patterns of the A- and B-rings of the 3-(2′-methoxybenzoyl)benzo[*b*]furan precursors. Owing to the structural similarities of the three products, erroneous assignments can be easily made if the anticipated products are characterized on the basis of 1D NMR spectroscopy only. There are a few reports on the synthesis of 2-unsubstituted benzoylbezofurans bearing a free hydroxy group at C-2′.[39,48] The first account by Wu and co-workers reported the synthesis of a series of SIRT1 inhibitors that included the 3-(2′-hydroxybenzoyl)benzo[*b*]furans **9**, **82**, and **83**. In their synthesis, compounds **82** and **83** were prepared by BBr_3 deprotection of the 3-(2′,5′-dimethoxybenzoyl)benzo[*b*]furan **21**, while compound **9** was prepared from the TBDMS-protected acetophenone (Scheme 5). The compounds were

Scheme 5. Synthesis of SIRT1 Inhibitors 9, 82, and 83[39a]



[a]Conditions: (a) DMF−DMA, DMF, 120 °C, 8 h; (b) for **9** and **21**: benzoquinone, HOAc, rt, 3 h; (c) for **82**: 2 M BBr_3, CH_2Cl_2, −78 °C, 2 h; (d) for **83**: 2 M BBr_3, CH_2Cl_2, −78 °C, 30 min, then 0 °C, 2 h.

characterized by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. Although the signals were not assigned, the resonances for the carbonyl carbon at δ_C 178.8 for **9**, δ_C 178.6 for **82**, and δ_C 178.8 for **83**[39] suggest that the structures of the three compounds should be revised to be the isoflavones **12**, **48**, and **49**, respectively (Figure 3). If the compounds were

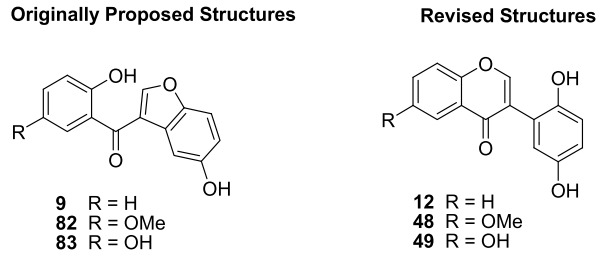


Figure 3. Proposed and revised structures for SIRT1 inhibitors.

benzoylbenzofurans, the ^{13}C NMR signals for the carbonyl groups would resonate around δ_C 190. This was indeed observed for compound **9** (δ_C 191.2) and for the benzoylbenzofurans **82** (δ_C 194.2) and **83** (δ_C 194.3) synthesized by $AlCl_3$ deprotection of compound **21** in the current investigation.

Finally, the synthetic utility of this method was demonstrated in a concise total synthesis of glaziovianin A (**1**). Glaziovianin A (**1**) was initially isolated from the leaves of the Brazilian tree *Ateleia glazioviana*, and it exhibited cytotoxicity against a panel of cancer cell lines.[7] More recently, an *O*-benzylated glaziovianin derivative was found to have stronger inhibition properties of microtubule polymerization than known *α*,*β*-tubulin inhibitors[33] such as colchicine and **1**, highlighting the importance of this scaffold in medicinal and synthetic chemistry. Since its discovery 10 years ago, only two synthetic routes have been reported for isoflavone **1**. The first synthesis by Hayakwa et al. proceeded via Suzuki−Miyaura coupling of a 3-halochromone and a boronic ester,[32] and the most recently developed method by Semenov and co-workers involved Cu-catalyzed cyclization of *β*-ketoaldehydes.[34]

The retrosynthetic analysis of glaziovianin A (**1**) is shown in Scheme 6. It is envisaged that coupling of the enaminone **87** with substituted benzoquinone **86** could form the benzoylbenzofuran **85** through a 1,4-Michael addition−cyclization

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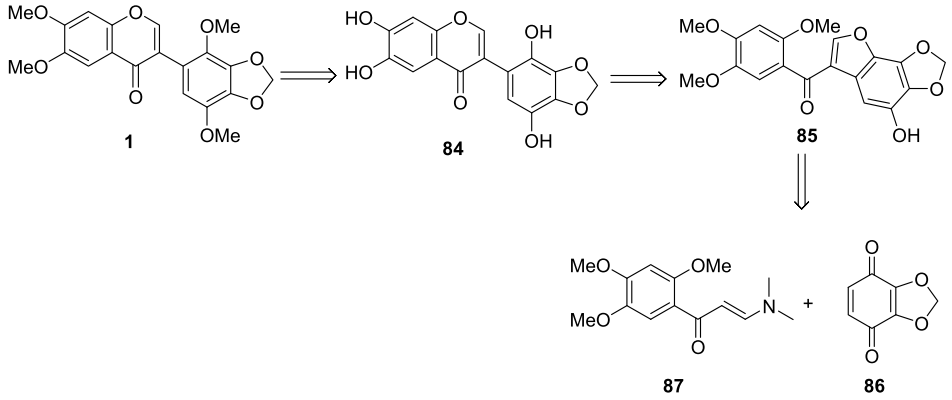
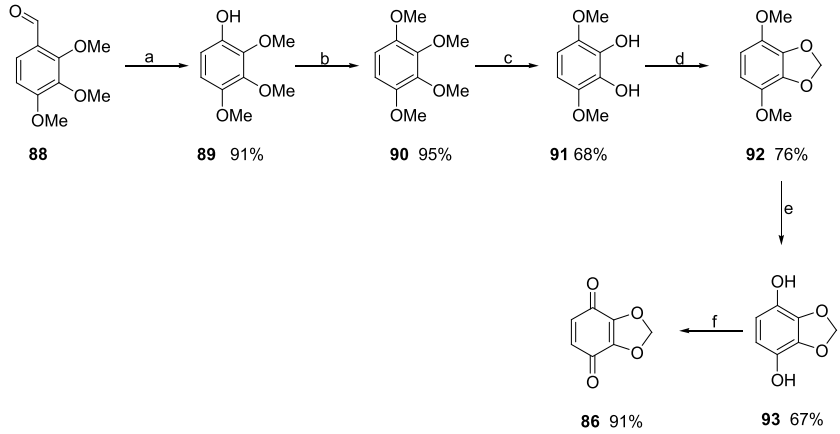
Scheme 6. Retrosynthetic Route for Glaziovianin A (1)

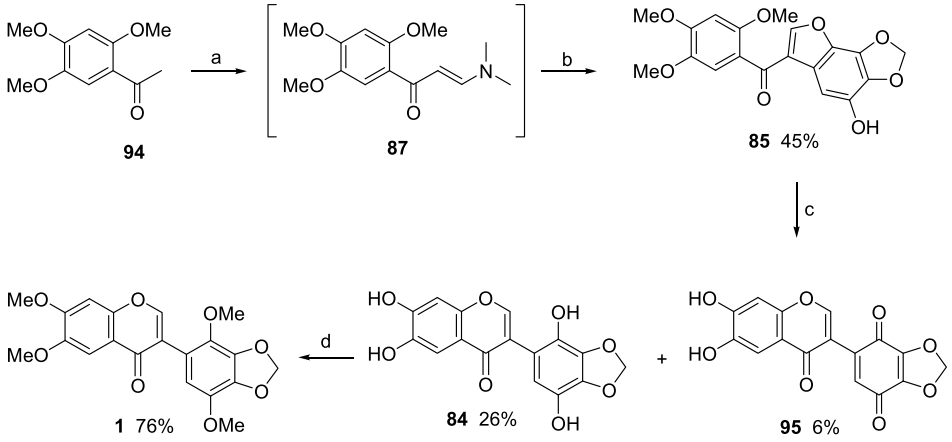


Scheme 7. Synthesis of 2,3-Methylenedioxy-1,4-benzoquinone[a]



[a]Conditions: (a) CH_2Cl_2, MeOH, H_2O_2, conc H_2SO_4, rt, 3 h; (b) DMF, K_2CO_3, MeI, 100 °C, 3 h; (c) CH_2Cl_2, $AlCl_3$, 0−rt, 18 h; (d) DMF, Cs_2CO_3, CH_2I_2, 100 °C, 6 h; (e) DMF, 98% TMSI, 80 °C, 36 h; (f) CH_2Cl_2, DDQ, rt, 1 h.

Scheme 8. Synthesis of Glaziovianin A (1)[a]



[a]Conditions: (a) DMF−DMA, 140 °C, 72 h; (b) HOAc, 2,3-methyleneoxy-1,4-benzoquinone (**86**), rt, 18 h; (c) DMF, TMSI, 80 °C; (d) DMF, K_2CO_3, MeI, 100 °C, 6 h.

reaction. Selective demethylation−cyclization of the benzoylbenzofuran **85** would afford the polyhydroxyisoflavone **84**, from which glaziovianin A (**1**) could be synthesized through *O*-methylation.

Although the quinone **86** is commercially available, larger quantities can be readily prepared from inexpensive commercial reagents. As depicted in Scheme 7, Dakin oxidation of trimethoxybenzaldehyde **88** gave trimethoxyphenol **89** in 91% yield. Subsequent methylation of the phenolic moiety with MeI afforded tetramethoxybenzene **90** in 95% yield, which upon selective demethylation with $AlCl_3$ afforded 3,6-dimethoxybenzene-1,2-diol (**91**) in 68% yield. This selective demethylation afforded good yields only when the reaction scale was below 4 g. When the reaction was performed

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with 10 g, only 32% of the target product was isolated, the bulk product being the mono-demethylated product. Acetalization of the diol **91** with methylene iodide afforded 4,7-dimethoxybenzo[*d*][2,3]dioxole (**92**) in 76% yield, which was subjected to selective demethylation with TMSI to afford the hydroquinone **93** in 67% yield. Subsequent oxidation of **93** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded benzoquinone **86** in 91% yield.

With the requisite benzoquinone **86** in hand, the next step was to prepare its coupling partner, the enaminoketone **87** (Scheme 8). Thus, condensation of 2,4,5-trimethoxyacetophenone (**94**) with DMF−DMA at elevated temperatures (150 °C) for 72 h afforded the enaminone **87**, which was used in the next step without further purification. The Michael addition−cyclization reaction of **87** with 2,3-methylenedioxy-1,4-benzoquinone (**86**) gave the benzoylbenzofuran **85** in 45% yield. Next, the demethylation−cyclization of the benzoylbenzofuran **85** using the newly established methodology was investigated. Treatment of the benzoylbenzofuran **85** with excess TMSI afforded the tetrahydroxyisoflavone **84** in 26% yield together with its oxidized derivative **95** in 6% yield. Subsequent methylation of the tetrahydroxyisoflavone **84** afforded the natural product **1** in 76% yield.

In conclusion, a method for the conversion of appropriately substituted benzoylbenzofurans to isoflavones has been developed. The procedure involves BBr_3- or TMSI-mediated tandem demethylation and ring-opening/cyclization of 3-(2′-methoxybenzoyl)benzo[*b*]furans to afford isoflavones. The synthetic utility of this method was demonstrated in the synthesis of various derivatives including the total synthesis of the potent inhibitor of α,β-tubulin polymerization, glaziovianin A (**1**). The development of the alternative synthetic route represents a significant advancement in the chemistry of isoflavonoids and benzofurans. Many biologically active natural isoflavonoids including coumaronochromones, rotenoids, coumestans, and pterocarpans contain the 2′,5′-oxygenation pattern. Therefore, the 2′,5′-dihydroxyisoflavones could serve as precursors to the synthesis of other complex isoflavonoids.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions requiring inert atmosphere were carried out under argon in oven-dried glassware unless otherwise specified. Unless otherwise noted, commercial solvents were used without prior purification. Solvents used for chromatographic purification were purchased from Radchem (Pty) Ltd. (Johannesburg, South Africa) or Pyramid Scientific (Pty) Ltd. (Pretoria, South Africa). Reagent-grade solvents and chemicals used for syntheses were purchased from Sigma-Aldrich (Pty) Ltd. (Johannesburg, South Africa) or Merck (Pty) Ltd. (Johannesburg, South Africa) and were used without further purification. All other solvents were purified and dried according to standard methods prior to use. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F_{254} aluminum-backed plates, and details of the eluents are described in each procedure. Plates were observed under UV light (254 nm) and/or developed in vanillin dip followed by heating. Column chromatography was run on silica gel (230−400 mesh) using the eluent system detailed for each procedure.

Synthesized compounds and key intermediates were characterized by IR, MS, and NMR data. The IR spectra were recorded on an Alpha Bruker Optics FT-IR spectrometer (neat), and all data were reported in wavenumbers. HR-MS (ESI-TOF) were recorded on a Waters UPLC coupled to a QTOF Synapt G2 spectrometer using electrospray ionization in the positive or negative mode. The NMR spectra were recorded at rt on a Bruker Avance III 400 MHz spectrometer or Bruker Ultra Shield 500 MHz spectrometer with a Prodigy probe. ^1H and ^{13}C NMR chemical shifts were referenced to residual protonated solvent peaks, which are δ_H 7.26, δ_C 77.0 for $CDCl_3$, δ_H 2.50, δ_C 39.52 for DMSO-d_6, δ_H 2.05, δ_C 29.84 for acetone-d_6, and δ_H 3.31, δ_C 49.0 for methanol-d_4. Chemical shifts are reported in ppm (δ) and spin−spin coupling constants (J) in hertz (Hz). The multiplicities of ^1H and ^{13}C NMR resonances are expressed by abbreviations: s (singlet), d (doublet), t (triplet), quartet (q), m (multiplet), and combinations thereof for highly coupled systems. DEPT and 2D NMR (HSQC, HMBC, NOESY, and COSY) spectra were used for the complete assignment of NMR signals when necessary. Chemical shifts that can be interchanged are marked with an asterisk (*) and superscripted letters.

General Procedure 1 (GP1) for the Synthesis of the Benzoylbenzofurans. The corresponding acetophenone (1 molar equiv) was dissolved in DMF−DMA (2.5 molar equiv) and stirred at 95−160 °C for 3−72 h under an argon atmosphere. After completion, the reaction mixture was concentrated in vacuo to give the crude enaminoketone, which was used in the next reaction without further purification.

To a stirred solution of crude enaminoketone was added the corresponding *p*-benzoquinone (1.2 molar equiv) and HOAc (3−6 molar equiv). After being stirred at rt for 12 h, the reaction mixture was quenched with ice-cold water and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed under vacuum. The residue was purified by silica gel flash column chromatography to afford the corresponding 3-benzoylbenzofuran.[36−38]

General Procedure 2 (GP2) for the Demethylation of Benzoylbenzofurans with BBr_3. To a stirred solution of a benzoylbenzofuran in anhydrous CH_2Cl_2, under an argon atmosphere at 0 °C, was added dropwise BBr_3 (3 molar equiv per methoxy). The reaction was allowed to warm to rt and stirred for 3−6 h. The reaction was quenched with 20% NaOH, followed by acidification to pH 2−3 with 1 M HCl, and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residual solid was concentrated onto silica gel and purified by column chromatography to afford the corresponding products.

General Procedure 3 (GP3) for the Demethylation of Benzoylbenzofurans with $AlCl_3$. To a stirred solution of a benzoylbenzofuran in anhydrous CH_2Cl_2, under an argon atmosphere at 0 °C, 8 molar equiv of $AlCl_3$ was added. The mixture was allowed to warm to rt and stirred for 12−18 h. The reaction was quenched with ice-cold water, followed by acidification to pH 2−3 with 1 M HCl and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residual solid was concentrated onto silica gel and purified by column chromatography.

In order to preserve Journal space, the ^1H and ^{13}C NMR and MS data of compounds **1**, **9**, **12**, **13**, **16**−**80**, **82**−**86**, **90**−**93**, and **95**, as well as some additional synthetic sequences, are shown in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00681.

Compound numbering adopted, physical data of some of the compounds, and copies of NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Veitch, N. C. *Nat. Prod. Rep.* **2007**, *24*, 417−464.
(2) Dewick, P. M.. *Medicinal Natural Products: A Biosynthetic Approach*; John Wiley & Sons: West Sussex, 2002; pp 8−166.
(3) War, A. R.; Paulraj, M. G.; Ahmad, T.; Buhroo, A. A.; Hussain, B.; Ignacimuthu, S.; Sharma, H. C. *Plant Signaling Behav.* **2012**, *7*, 1306−1320.
(4) Wollenweber, E. In *The Flavonoids: Advances in Research*; Harborne, J. B.; Mabry, T. J., Eds.; Springer-Science: Dordrecht, 1982; pp 189−193.
(5) Chan, H.-W., Rice, G. E., Mitchell, M. D. In *Isoflavones: Chemistry, Analysis, Function and Effects*; Preedy, V. R., Ed.; Royal Society of Chemistry: Cambridge, 2013; pp 115−126.
(6) Veitch, N. C. *Nat. Prod. Rep.* **2013**, *30*, 988−1027.
(7) Yokosuka, A.; Haraguchi, M.; Usui, T.; Kazami, S.; Osada, H.; Yamori, T.; Mimaki, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3091−3094.
(8) Shou, Q. Y.; Tan, Q.; Shen, Z. W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3389−3391.
(9) Lo, W.-L.; Chang, F.-R.; Hsieh, T.-J.; Wu, Y.-C. *Phytochemistry* **2002**, *60*, 839−845.
(10) Lo, W.-L.; Wu, C.-C.; Chang, F.-R.; Wang, W.-Y.; Khalil, A. T.; Lee, K.-H.; Wu, Y.-C. *Nat. Prod. Res.* **2003**, *17*, 91−97.
(11) Chin, Y.-W.; Mdee, L. K.; Mbwambo, Z. H.; Mi, Q.; Chai, H.-B.; Cragg, G. M.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2006**, *69*, 1649−1652.
(12) Deesamer, S.; Kokpol, U.; Chavasiri, W.; Douillard, S.; Peyrot, V.; Vidal, N.; Combes, S.; Finet, J.-P. *Tetrahedron* **2007**, *63*, 12986−12993.
(13) Burali, C.; Desideri, N.; Stein, M. L.; Conti, C.; Orsi, N. *Eur. J. Med. Chem.* **1987**, *22*, 119−123.
(14) Alberola, A.; Andrés, C.; Ortega, A. G.; Pedrosa, R.; Vicente, M. *J. Heterocycl. Chem.* **1986**, *23*, 1781−1783.
(15) Selepe, M. A.; Drewes, S. E.; van Heerden, F. R. *J. Nat. Prod.* **2010**, *73*, 1680−1685.
(16) Hastings, J. M.; Hadden, M. K.; Blagg, B. S. *J. Org. Chem.* **2008**, *73*, 369−373.
(17) Balasubramanian, S.; Nair, M. G. *Synth. Commun.* **2000**, *30*, 469−484.
(18) Kim, Y.-W.; Brueggemeier, R. W. *Tetrahedron Lett.* **2002**, *43*, 6113−6115.
(19) Sato, S.; Hiroe, K.; Kumazawa, T.; Jun-Ichi, O. *Carbohydr. Res.* **2006**, *341*, 1091−1095.
(20) Bhrara, S. C.; Jain, A. C.; Seshadri, T. R. *Tetrahedron* **1965**, *21*, 963−967.
(21) Bhrara, S. C.; Jain, A. C.; Seshadri, T. R. *Tetrahedron* **1964**, *20*, 1141−1149.
(22) McKillop, A.; Hunt, J. D.; Taylor, E. C.; Kienzle, F. *Tetrahedron Lett.* **1970**, *11*, 5275−5280.
(23) Ollis, W.; Ormand, K.; Redman, B.; Roberts, R.; Sutherland, I. *J. Chem. Soc. C* **1970**, 125−128.
(24) Li, S.-R.; Chen, P.-Y.; Chen, L.-Y.; Lo, Y.-F.; Tsai, I.-L.; Wang, E.-C. *Tetrahedron Lett.* **2009**, *50*, 2121−2123.
(25) Hoshino, Y.; Miyaura, N.; Suzuki, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3008−3010.
(26) Felpin, F.-X.; Lory, C.; Sow, H.; Acherar, S. *Tetrahedron* **2007**, *63*, 3010−3016.
(27) Klier, L.; Bresser, T.; Nigst, T. A.; Karaghiosoff, K.; Knochel, P. *J. Am. Chem. Soc.* **2012**, *134*, 13584−13587.
(28) Zhang, Z.; Qiao, J.; Wang, D.; Han, L.; Ding, R. *Mol. Diversity* **2014**, *18*, 245−251.
(29) Granados-Covarrubias, E. H.; Maldonado, L. A. *Tetrahedron Lett.* **2009**, *50*, 1542−1545.
(30) Li, Q.-L.; Liu, Q.-L.; Ge, Z.-Y.; Zhu, Y.-M. *Helv. Chim. Acta* **2011**, *94*, 1304−1309.
(31) Selepe, M. A.; Drewes, S. E.; van Heerden, F. R. *Tetrahedron* **2011**, *67*, 8654−8658.
(32) Hayakawa, I.; Ikedo, A.; Chinen, T.; Usui, T.; Kigoshi, H. *Bioorg. Med. Chem.* **2012**, *20*, 5745−5756.
(33) Hayakawa, I.; Shioda, S.; Chinen, T.; Hatanaka, T.; Ebisu, H.; Sakakura, A.; Usui, T.; Kigoshi, H. *Bioorg. Med. Chem.* **2016**, *24*, 5639−5645.
(34) Semenov, V. V.; Tsyganov, D. V.; Semenova, M. N.; Chuprov-Netochin, R. N.; Raihstat, M. M.; Konyushkin, L. D.; Volynchuk, P. B.; Marusich, E. I.; Nazarenko, V. V.; Leonov, S. V.; Kiselyov, A. S. *J. Nat. Prod.* **2016**, *79*, 1429−1438.
(35) Flynn, B. L.; Gill, G. S.; Grobelny, D. W.; Chaplin, J. H.; Paul, D.; Leske, A. F.; Lavranos, T. C.; Chalmers, D. K.; Charman, S. A.; Kostewicz, E.; Shackleford, D. M.; Morizzi, J.; Hamel, E.; Jung, M. K.; Kremmidiotis, G. *J. Med. Chem.* **2011**, *54*, 6014−6027.
(36) Mukhanova, T. I.; Alekseeva, L. M.; Granik, V. G. *Khim. Geterotsikl. Soedin.* **1990**, 888−891.
(37) Trofimov, F. A.; Mukhanova, T. I.; Grinev, A. N.; Shvedov, V. I. *Zh. Org. Khim.* **1967**, *3*, 2185−2188.
(38) Cheng, X.-M.; Liu, X.-W. *J. Comb. Chem.* **2007**, *9*, 906−908.
(39) Wu, J.; Li, Y.; Chen, K.; Jiang, H.; Xu, M.-H.; Liu, D. *Eur. J. Med. Chem.* **2013**, *60*, 441−450.
(40) Guo, R.-Y.; Sun, L.; Pan, X.-Y.; Yang, X.-D.; Ma, S.; Zhang, J. *Chem. Commun.* **2018**, *54*, 12614−12617.
(41) Weaver, M. G.; Pettus, T. R. R. In *Comprehensive Organic Synthesis*; Knochel, P.; Molander, G. A., Eds.; Elsevier: Amsterdam, 2014; pp 375.
(42) Kim, A. I.; Rychnovsky, S. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 1267−1270.
(43) Munday, R. *Free Radical Biol. Med.* **1999**, *26*, 1475−1479.
(44) Burfeindt, J.; Patz, M.; Müller, M.; Mayr, H. *J. Am. Chem. Soc.* **1998**, *120*, 3629−3634.
(45) Gabsi, W.; Boubaker, T.; Goumont, R. *Int. J. Chem. Kinet.* **2014**, *46*, 711−717.
(46) Mayr, H.; Kempf, B.; Ofial, A. R. *Acc. Chem. Res.* **2003**, *36*, 66−77.
(47) Mayr, H.; Patz, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 938−957.
(48) Bose Bathula, S.; Khagga, M.; Venkatasubramanian, H. *Lett. Org. Chem.* **2017**, *14*, 353−360.

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