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Efficient Synthesis of Frutinone A and Its Derivatives through Palladium-Catalyzed C–H Activation/Carbonylation

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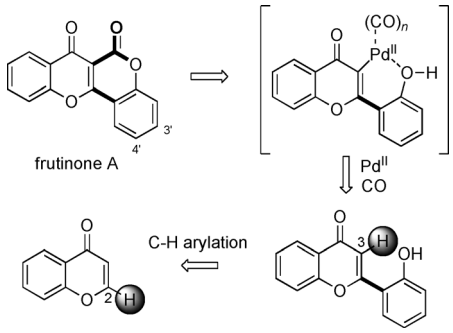
Abstract: Frutinone A, a biologically active ingredient of an antimicrobial herbal extract, demonstrates potent inhibitory activity towards the CYP1A2 enzyme. A three-step total synthesis of frutinone A with an overall yield of 44% is presented. The construction of the chromone-annelated coumarin core was achieved through palladium-catalyzed C–H carbonylation of 2-phenolchromones. The straightforward synthetic route allowed facile substitutions around the frutinone A core and thus rapid exploration of the structure–activity relationship (SAR) profile of the derivatives. The inhibitory activity of the synthesized frutinone A derivatives were determined for CYP1A2, and ten compounds exhibited one-to-two digit nanomolar inhibitory activity towards the CYP1A2 enzyme.

Frutinone A (**1a**), isolated from the leaves and root bark of *Polygala fruticosa*, possesses a chromonocoumarin structural scaffold. Frutinone A shows various biological activities, including antibacterial, antioxidant, and potent cytochrome P450 1A2 (CYP1A2) inhibition.[1] This intriguing molecule continues to attract considerable attention from research groups, and several synthetic approaches to obtain the frutinone scaffold have been developed.[2] However, previously developed synthetic routes to frutinone A suffer from the drawbacks of having multiple steps and low-to-moderate yields. A potential alternative synthetic route to frutinone A would be to use C–H functionalization,[3] which enables the construction of complicated target molecules in fewer reaction steps without pre-functionalization of the starting materials. To identify more potent CYP1A2 inhibitors, we were particularly interested in an efficient synthesis of frutinone A and its derivatives by exploring C–H bond functionalization, which involves the use of the innate nucleophilic characteristics of chromones for the efficient synthesis of frutinone A derivatives.[4] Our retrosynthetic strategy for frutinone A is illustrated in Scheme 1. In view of recent advances in C–H bond carbonylation,[5] we envisaged that the chromone-annelated coumarin architecture might be constructed from 2-phenylflavone through a palladium-catalyzed C–H activation/carbonylation process. Through these efforts, we established an efficient palladium catalytic protocol for the facile construction of a chromone-annelated coumarin motif. Herein, we report a straightforward synthetic approach that is broadly applicable to readily accessible chromone systems for the synthesis frutinone A derivatives, and evaluate the biological characteristics of the derivatives obtained.



Scheme 1. Synthetic approach to frutinone A.

To construct the chromonocoumarin framework, we initially focused on the direct C–H bond functionalization/carbonylation reaction. The feasibility of this process was tested by using the reaction of 2-phenolchromone **1a** as a model substrate in the presence of the Pd^{II} catalyst under 1 atm CO; representative screening data are listed in Table 1. We found that the reactions that used $Cu(OAc)_2$ as an oxidant and Na_2CO_3 as a base at 110 °C provided a noticeable product, albeit only in 6% yield (Table 1, entry 1). Of the palladium sources tested, $Pd(OAc)_2$ displayed the best catalytic reactivity. Further investigations of the reaction conditions revealed that the choice of solvent was critical for the efficiency of the reaction, with no detectable desired product being obtained in polar solvents, such as 1,4-dioxane. However, the product yield dramatically increased to

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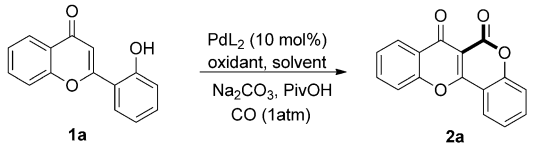
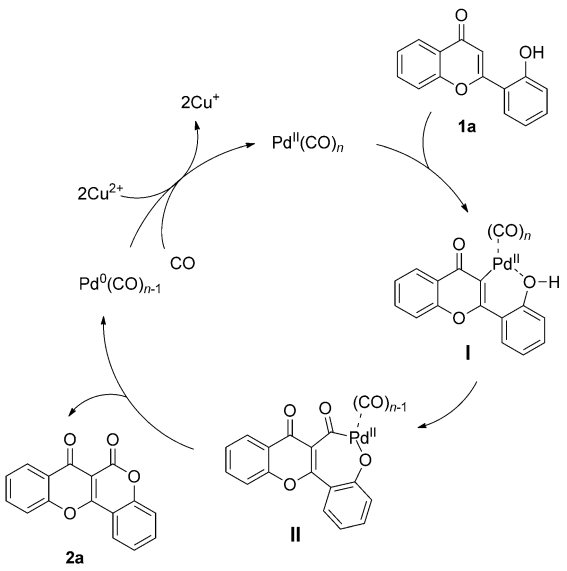
Table 1. Optimization of the reaction conditions.[a]



Entry	Catalyst (10 mol %)	Oxidant (2 equiv)	Solvent	T [°C]	Yield [%]
1	$Pd(OAc)_2$	$Cu(OAc)_2$	toluene	110	6
2	$Pd(OAc)_2$	$Cu(OAc)_2$	1,4-dioxane	110	–
3	$Pd(OAc)_2$	$Cu(OAc)_2$	xylene	110	44
4	$Pd(OAc)_2$	$Cu(OAc)_2$	mesitylene	110	61
5	–	$Cu(OAc)_2$	mesitylene	110	–
6	$Pd(OAc)_2$	–	mesitylene	110	7
7	$Pd(OAc)_2$	AgOAc	mesitylene	110	17
8	**$Pd(OAc)_2$**	**$Cu(OAc)_2$**	**mesitylene**	**125**	**86**
9	$Pd(OPiv)_2$	$Cu(OAc)_2$	mesitylene	125	83
10[b]	$Pd(OAc)_2$	$Cu(OAc)_2$	mesitylene	125	42

[a] Reactions were conducted with **1a**, PdL_2 (10 mol %), and oxidant (2 equiv), Na_2CO_3 (2 equiv), and PivOH (4 equiv) in solvent at 125 °C for 3 h under CO (1 atm). [b] Conditions: $Pd(OAc)_2$ (2.5 mol %) under otherwise identical conditions. Piv = pivaloyl.

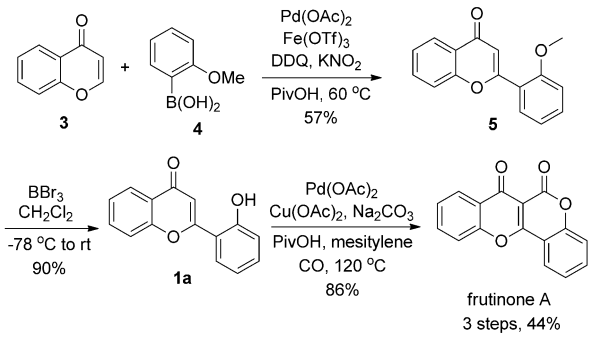


Scheme 2. Proposed mechanism for the present reaction.

44 % in xylene (Table 1, entry 3) and 61 % in mesitylene (Table 1, entry 4). In the absence of $Pd(OAc)_2$, the intramolecular C–O coupling reaction preferentially proceeded to provide flavone-fused benzofuran in 42 % yield[6] and no desired product was obtained. The oxidizing agent was also critical to the efficiency of the transformation, with the desired product (**2a**) being obtained in only 7 % yield in the absence of $Cu(OAc)_2$ (Table 1, entry 6). A variety of oxidizing agents, including Cu^{II}, benzoquinone (BQ), $K_2S_2O_8$, (2,2,6,6-tetramethyl-piperidin-1-yl)oxy (TEMPO), and MnO_2 were evaluated, and $Cu(OAc)_2$ was found to be the most effective and economical oxidant. Under the optimized reaction conditions, the C–H activation/carbonylation of **1a** (1 equiv) in the presence of $Pd(OAc)_2$ (10 mol %), $Cu(OAc)_2$ (2 equiv), Na_2CO_3 (2 equiv), and PivOH (4 equiv) in mesitylene at 125 °C under 1 atm CO afforded product **2a** in the highest yield (86 %). Under these reaction conditions, no benzofuran side product from an intramolecular C–O coupling reaction was observed.

A plausible mechanism for the C–H activation/carbonylation process is outlined in Scheme 2. Initially, six-membered palladacycle **I** is formed by the chelation of the hydroxyl group of **1a** with the CO-ligated Pd^{II} complex, followed by C–H activation through electrophilic cyclopalladation at the C3 position of the flavone derivative. Then, the migratory insertion of coordinated CO into the aryl–Pd bond forms seven-membered palladacycle **II**. Next, palladacycle **II** transforms desired product **2a** and Pd^0 species through reductive elimination. Finally, Pd^0 is oxidized in the presence of Cu^{II} to regenerate Pd^{II} and complete the catalytic cycle.

Having determined the optimized C–H activation/carbonylation conditions, we next turned our attention to the efficient and step-economical synthetic route to frutinone A. Recently, our group reported a practical method for the palladium-catalyzed 1,4-addition of arylboronic acids to chromones in the presence of a catalytic amount of $Fe(OTf)_2$, which thereby gave a variety of flavone analogues as major products under mild conditions.[7] This protocol was subjected to a straightforward synthetic route to frutinone A and its derivatives, as outlined in Scheme 2. Thus, required 2-phenol chromone **1a** was conveniently prepared by palladium-catalyzed 1,4-addition of methoxyphenylboronic acid **4** to chromone **3**, followed by the demethylation of **5** with BBr_3. Next, a palladium-catalyzed C–H activation/carbonylation reaction was applied and frutinone A was obtained by starting from commercially available chromone **3** in a total yield of 44 % over three steps (see Scheme 3).



Scheme 3. Total synthesis of frutinone A; DDQ = 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone.

CYP1A2 has been shown to be involved in the activation of carcinogens and mutagens by controlling drug metabolism, which indicates that CYP1A2 inhibition could potentially be exploited clinically in areas such as cancer prevention.[8] With the goal of identifying more potent CYP1A2 inhibitors, we investigated the binding modes of frutinone A in the active site of CYP1A2 in a comparative fashion.

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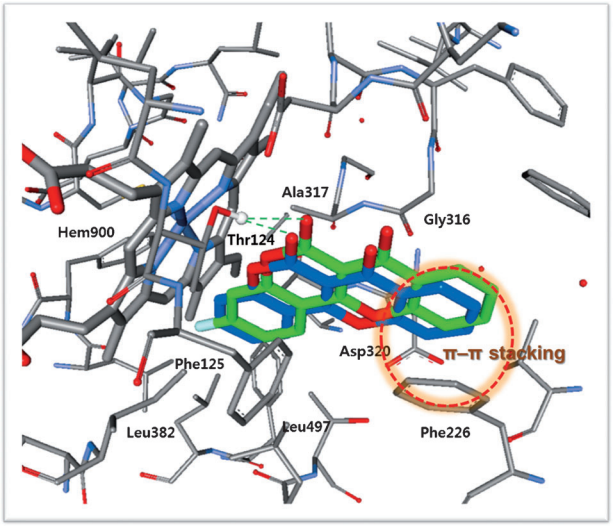
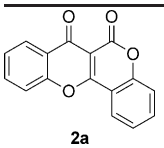
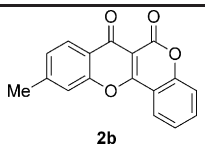
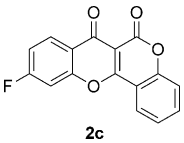
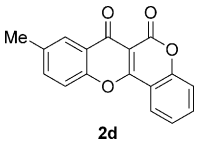
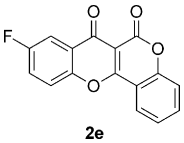
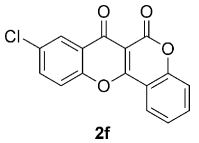
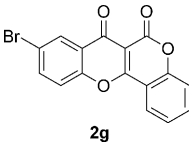
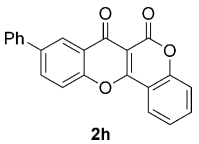
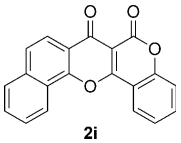
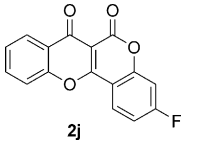
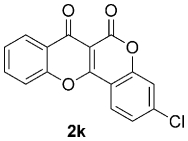
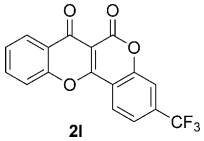
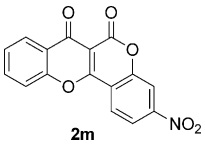
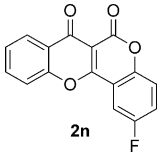
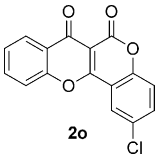
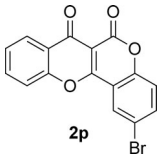
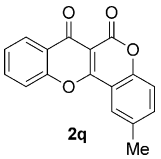
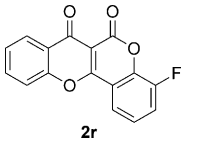


Figure 1. Calculated binding mode of frutinone A (blue) and compound **2j** (green) in the active site of CYP1A2. Heme is shown as dark grey sticks in the top-left corner. Each dotted line indicates a hydrogen bond.

Figure 1 shows the lowest-energy conformation of frutinone A in the active site of CYP1A2, as calculated by using the modified AutoDock program.[9] From the overall structural features derived from the docking simulations, the carbonyl oxygen of the lactone ring appeared to form a hydrogen bond with the side-chain hydroxyl group of Thr124. Frutinone A could be further stabilized in the active site of CYP1A2 through a π-stacking interaction with the Phe226 residue, and hydrophobic interactions with the side chains of Ala317, Gly316, Asp320, Leu497, Phe125, Leu382, and Hem900. The docking studies indicated that the presence of an additional small substituent on the frutinone A core would be tolerated in its function on CYP1A2 without causing an unfavorable steric clash. For example, frutinone A derivative **2j** exhibited similar configurations and comparable interactions with the amino acid residues in the active site (Figure 1). Moreover, **2j** appears to be more deeply located in the hydrophobic pocket, which allows a stronger interaction with the side chain of Phe226 through π–π stacking. The structural analysis suggested the need to widen the exploration of analogues that incorporate small groups around the frutinone A scaffold.

Based on our structural analysis, we planned to install a variety of small-sized substituents around the frutinone A core with a view to expanding the SAR profile in the hope of identifying derivatives with enhanced potency. The frutinone A scaffold could be easily equipped with an additional substituent by using the newly developed synthetic approach. Substituted 2-phenol chromones were efficiently prepared in a two-step sequence, as shown in Scheme 2. Subsequently, the palladium-catalyzed C–H activation/carbonylation reaction of a range of 2-phenol chromones successfully provided the desired frutinone A derivatives in moderate-to-good yields, as illustrated in Table 2.

Table 2. Substrate scope.[a]

Compound	Yield[b] [%]	Compound	Yield[b] [%]
 **2a**	86	 **2b**	75
 **2c**	89	 **2d**	68
 **2e**	74	 **2f**	63
 **2g**	52	 **2h**	76
 **2i**	63	 **2j**	56
 **2k**	77	 **2l**	62
 **2m**	43	 **2n**	65
 **2o**	56	 **2p**	48
 **2q**	68	 **2r**	60

[a] Conditions: Flavone (1 equiv), $Pd(OAc)_2$ (10 mol%), $Cu(OAc)_2$ (2 equiv), Na_2CO_3 (2 equiv), and PivOH (4 equiv) in mesitylene under CO (1 atm) at 120–130 °C for 3–21 h. [b] Yield of the isolated product.

The IC_{50} values of the synthesized derivatives were determined for CYP1A2.[10] Of the derivatives tested, ten compounds were found to have one-to-two digit nanomolar inhibitory activities towards CYP1A2, as summarized in Table 3. Notably, compound **2j**, which contains a fluoro group at the 3′ position, showed potent inhibitory activity towards CYP1A2 (IC_{50} = 2.65 nM), whereas substitution with

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Table 3. Inhibitory activities potency profiles over CYP1A2.

Compound	CYP1A2 IC_{50} [nM]	Compound	CYP1A2 IC_{50} [nM]
2a	5.33	**2j**	2.65
2b	51.2	**2k**	44.6
2c	14.6	**2n**	12.9
2d	52.0	**2o**	11.0
2e	45.3	**2r**	12.6

the bulkier chloro group at this position led to reduced activity (**2k**: IC_{50} = 44.6 nM). Note that compound **2o**, with a chloro group at the 4′ position, exerted potent effects on CYP1A2 (IC_{50} = 11.0 nM).

In summary, we have developed an efficient method for synthesizing the chromone-annelated coumarin motif through palladium-catalyzed C–H activation/carbonylation of 2-phenolchromone derivatives in the presence of CO. The developed reaction was subjected to the concise synthesis of a variety of frutinone A derivatives. Several compounds inhibited the activity of the CYP1A2 enzyme in the nanomolar range. Further studies aimed at broadening the synthetic application toward other heterocycles are in progress.

Experimental Section

*Representative Experimental Procedure (**2a**)*

2-Phenol chromone **1a** (0.1 mmol), $Pd(OAc)_2$ (10 mol%), $Cu(OAc)_2$ (2 equiv), Na_2CO_3 (2 equiv), and PivOH (4 equiv) were combined in mesitylene (0.1 M) in a 20 mL Schlenk tube. Then, a balloon charged with CO gas was connected to the Schlenk tube. The reaction mixture was heated at 125 °C for 3 h, and monitored by TLC with ethylacetate/*n*-hexane (1:1) as the mobile phase. After cooling to RT, the mixture was quenched with water (10 mL) and the aqueous phase was extracted with CH_2Cl_2 (3×20 mL). The organic phases were combined and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (ethylacetate/*n*-hexane 1:1) on silica gel to give the desired product (22.7 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34 (dd, J = 8.0, 1.7 Hz, 1H), 8.21 (dd, J = 8.0, 1.6 Hz, 1H), 7.83–7.70 (m, 2H), 7.62 (dd, J = 8.4, 1.0 Hz, 1H), 7.50 (ddd, J = 8.1, 7.2, 1.1 Hz, 1H), 7.45 (ddd, J = 8.1, 7.3, 1.1 Hz, 1H), 7.39 ppm (dd, J = 8.4, 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.1, 165.1, 156.4, 154.5, 154.4, 135.7, 134.9, 127.0, 126.7, 125.0, 124.6, 124.3, 118.0, 117.5, 113.4, 105.2 ppm; HRMS ESI: m/z calculated for $C_{16}H_8NaO_4^+$: 287.0320, found: 287.0289 $[M+Na]^+$.

Acknowledgements

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Keywords: carbonylation · C–H functionalization · frutinone A · palladium · structure–activity relationships

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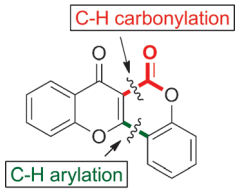
COMMUNICATION

Bearing frutinone: A three-step total synthesis of frutinone A (see scheme) with an overall yield of 44% is presented. The construction of the chromone-annelated coumarin core was achieved through palladium-catalyzed C–H carbonylation of 2-phenolchromones. The straightforward synthetic route allowed facile substitutions around the frutinone A core and thus rapid exploration of the structure–activity relationship profile of the derivatives.



frutinone A

C–H Activation

Yongje Shin, Changho Yoo, Youngtaek Moon, Yunho Lee, Sungwoo Hong** _____ ▮▮▮▮–▮▮▮▮

Efficient Synthesis of Frutinone A and Its Derivatives through Palladium-Catalyzed C–H Activation/Carbonylation

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