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Synthesis and Anticoagulant Activity of C3- and *O*-alkylated 4-Hydroxycoumarin Derivatives

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Short Communication

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ABSTRACT

C3- and *O*-alkylated 4-hydroxycoumarin derivatives synthesized from reusable solid superacid catalyst, namely, sulfated tin oxide (STO) in acetic acid under reflux conditions with good yields and well characterized by IR, $^{1}H/^{13}C$ NMR and mass spectrometry. The synthesized compounds were evaluated for anticoagulant activity. Among the compounds tested for anti-coagulant activity, compounds **3b**, **3c, 3d** and **3g** showed significant activity compared to that of standard.

INTRODUCTION

Coumarin is a privileged scaffold among heterocycles and is known to possess a wide range of biological activities including antibiotic, anti-malarial, antifungal, anti-viral, and cytotoxic [1,2,3,4,5,6,7,8].

In particular, the 4-hydroxycoumarins and its derivatives (3-alkylated) have evoked a great deal of interest due to their utility as 'anticoagulant rodenticides as well as antithrombotic agents' such as warfarin, brodifacoum, difethialone, bromadiolone, coumatetralone, and flocoumafen [9] (**Figure 1**) and also as nonpeptide human immunodeficiency virus (HIV) protease inhibitors [10]. The C3 or *O*-alkylation of 4-hydroxycoumarin is undoubtedly one of the most important and challenging reactions in synthetic chemistry due to its pharmaceutical utility as mentioned above and also can be diversified to synthesize 3,4-substitued compounds [11,12,13,14].

In continuation of our interest in developing novel synthetic methodologies, particularly carbon-carbon, carbon-heteroatom bond formations to synthesize pharmaceutically relevant heterocycles [15], we have very recently reported SO_4^{2-}/SnO_2-catalyzed C3-alkylation of 4-hydroxycoumarin with secondary benzyl alcohols and *O*-alkylation with *O*-acetyl compounds [16].

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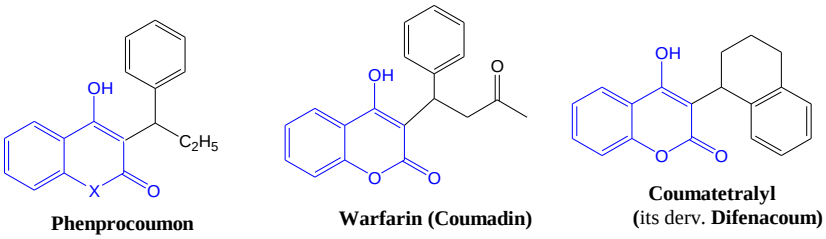
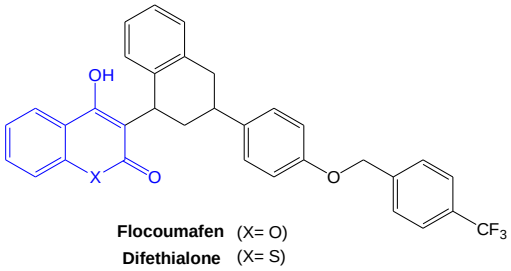
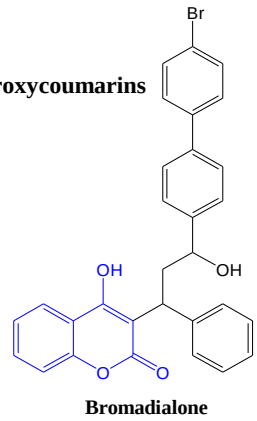


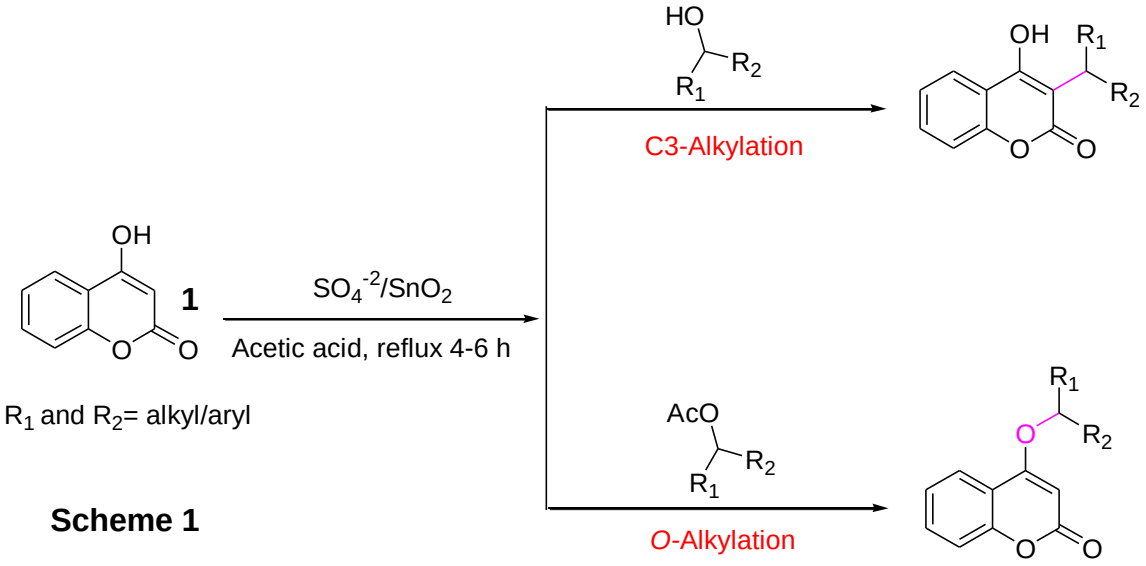
Figure 1. Prominet 3-(benzyl)-substituted 4-hydroxycoumarins





Chemistry

With the optimized reaction conditions in hand (10 mol% STO, AcOH, reflux), we then evaluated the scope of the benzylation of 4-hydroxycoumarin **1** using a variety of structurally divergent reactants (**Scheme 1**), When primary benzyl alcohols were used, they failed to give the expected product. These results clearly demonstrate that the direct C3-alkylation of 4-hydroxycoumarin was successful only with secondary benzylic alcohols. We obtained the corresponding C3-alkylated products in 70-81% yields, after 6 h (entries 1-3, Table 1). When nitro group containing benzylic alcohol was introduced instead of bromo- or methoxy, the reaction did not proceed at all.



Scheme 1

After successful C3-alkylation of 4-hydroxycoumarin with secondary benzyl alcohols, we turned our attention to test the feasibility of *O*-alkylation using secondary benzyl acetates (prepared instantly for the purpose) reacting with 4-hydroxycoumarin under the above optimized conditions to generate novel compounds (entries 4-7**,** Table 1) moderate to good yields (72-85%) in the specified time. Enolic hydroxyl group is activated by Sn metal *via* Lewis acid catalysis to make the 3-position more nucleophilic. Where as, in case of *O*-alkylation, activation of carbonyl functionality of acetate making it as leaving group by Sn *via* Lewis acid catalysis and then the formed stabilized carbocation reacts with enolic hydroxide leaving AcOH as byproduct.

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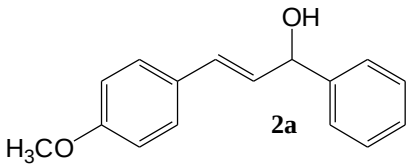
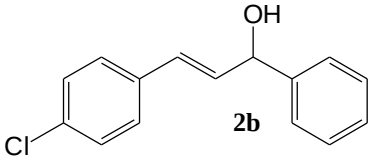
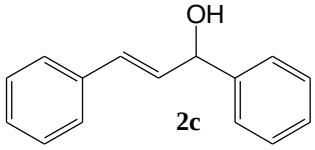
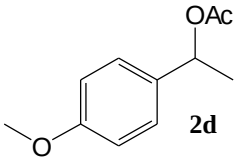
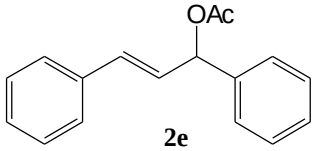
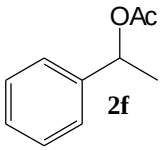
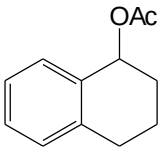
Table 1. *C*- and *O*-alkylation of 4-hydroxycomarin (**1**) with corresponding alcohols and acetates

Entry	Alcohol/Acetate	Product	Time (h)	Yield (%)[a]
1	 **2a**	**3a**	**6**	**70**
2	 **2b**	**3b**	**6**	**78**
3	 **2c**	**3c**	**6**	**81**
4	 **2d**	**3d**	**4**	**78**
5	 **2e**	**3e**	**5**	**73**
6	 **2f**	**3f**	**4**	**67**
7	 **2g**	**3g**	**6**	**82**

[a] Isolated yields after column chromatography.

EXPERIMENTAL SECTION

STO was prepared according to the literature report. All melting points were determined on an Electrothermal Gallenkamp apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini Spectrometer 300 MHz. IR spectra were recorded on Nicolet Fourier Transform spectrometer. Mass spectra were obtained on a 7070H or VG Autospec Mass spectrometer using LSIMS technique. Thin-layer chromatography (TLC) was performed on GF-25U (Anal. Tech) plates and silica gel glass-backed plates. Routine column chromatography was conducted using silica gel 100-200 mesh.

General experimental procedure for the C3-alkylation and O-alkylation of 4-hydroxycoumarins

To a mixture of 4-hydroxycoumarin (1, 1.0 mmol) and corresponding secondary benzyl alcohol/ secondary *O*-acetyl compound (2a-g, 1.1 mmol) in acetic acid (10 mL), STO (0.1 mmol) was added and the reaction mixture was stirred for the given time (see Table 1) at

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reflux temperature. After completion of the reaction (monitored by TLC), the reaction mixture was added into water. Adjusted to pH neutral with sodium carbonate and extracted in ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was purified by silica gel column with petroleum ether/ethyl acetate (1:3) as eluent to afford the corresponding C3-alkylated/*O*-alkylated 4-hydroxycoumarin (**3a-g**).

Characterization Data for New Compounds:

3-((*E*)-3-(4-chlorophenyl)-1-phenylallyl)-4-hydroxy-2*H*-chromen-2-one (**3b**): Pale yellow solid, mp: 168-171°C. IR (KBr): ν 3327, 1674, 1626, 1611, 1494, 1393, 1200, 756 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.81-7.72 (m, 2H), 7.57-7.28 (m, 11H), 6.78-6.68 (m, 1H), 6.48 (d, *J*=16.4 Hz, 1H), 5.46 (d, *J*=6 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 160.9, 152.5, 140.1, 136.2, 133.7, 132.2, 1130.0, 128.7, 128.2, 127.9, 127.6, 126.6, 124.1, 123.2, 116.5, 115.8, 106.5, 44.0 ppm. MS (ESI): *m/z* (rel. abund.%) 389 (M^+, 100), 391 (M^+, 30) ($[M+1]^+$).

3-[(*E*)-1,3-diphenyl-2-propenyl]-4-hydroxy-2*H*-2-chromenone (**3c**). White solid. mp: 155-157 °C. IR (KBr): 3330, 1671, 1624, 1610, 1494, 1392, 1201, 754 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.50 (d, *J*=6.5 Hz, 1H), 6.55 (d, *J*=16.0 Hz, 1H), 6.81(dd, *J*=6.0, 16.0 Hz, 1H), 7.22-7.44 (m, 12H), 7.50-7.55 (m, 1H), 7.86 (dd, *J*=6.8, 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.59, 161.26, 152.78, 140.00, 136.42, 133.83, 132.29, 129.29, 128.77, 128.43, 128.14, 128.11, 127.63, 126.67, 124.18, 123.33, 116.65, 116.09, 106.75, 44.14. MS (ESI): *m/z* (rel. abund.%) 355 (M^+, 100).

4-(1-(4-methoxyphenyl) ethoxy)-2H-chromen-2-one (**3d**): Off white solid. mp: 180-184 °C. IR (KBr): 3342, 1673, 1628, 1514, 1249 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.70 (dd, *J* = 8.8Hz, 1H), 7.48-7.52 (m, 2H), 7.41 (d, *J* =11.2 Hz, 1H), 7.26-7.22 (m, 2H), 6.99 (d, *J* =4 Hz, 2H), 6.04 (s, 1H), 4.65 (q, *J* =10 Hz, 1H), 3.79 (s, 3H), 1.60 (d, *J* = 9.6 Hz, 3H). ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 159.8, 159.1, 152.3, 133.1, 131.7, 128.5, 123.8, 122.6, 116.2, 116.1, 114.9, 110.0, 55.3, 33.6, 16.8 ppm. MS (ESI): *m/z* (rel. abund.%) 297.2 ($[M+1]^+$, 100).

4-((*E*)-1,3-diphenylallyloxy)-2H-chromen-2-one (**3e**): Pale yellow solid, mp: 132-136 °C. IR (KBr): ν 1678, 1626, 1613, 1501, 1394, 1203, 757 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, *J* =7.2 Hz, 1H), 7.55 (t, *J* =8 Hz, 1H), 7.24-7.64 (m, 12H), 6.94 (br, s 1H), 6.67 (dd, *J* =6.4, 9.6 Hz, 1H), 6.52 (d, *J* =16.4 Hz, 1H), 5.47 (d, *J* = 5.6 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.3, 161.5, 152.4, 139.7, 136.3, 133.9, 132.4, 129.2, 128.7, 128.2, 128.7, 127.7, 126.4, 124.4, 123.1, 116.5, 115.7, 106.4, 43.5 ppm. MS (ESI): *m/z* (rel. abund.%) 355.0 ($[M+1]^+$,100).

4-(1-Phenylethoxy)-2H-chromen-2-one (**3f**). Off white solid; mp: 214-218 °C. IR (KBr): ν 1669, 1621, 1492, 1401, 1218, 1168, 741 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, *J* =12.4 Hz, 1H), 7.64-7.43 (m, 5H), 7.64-7.42 (m, 2H), 7.23 (dd, *J* =10.8 Hz, 1H), 5.99 (br s, 1H), 4.74 (q, *J*=9.6 Hz, 1H), 1.68 (d, *J*= 9.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 160.0, 152.6, 141.8, 132.1, 129.8, 127.7, 127.5, 123.9, 123.0, 116.3, 116.2, 110.3, 34.8, 16.8 ppm. MS (ESI): *m/z* (rel. abund.%) 267.3 ($[M+1]^+$, 100).

4-(1, 2, 3, 4-tetrahydronaphthalen-4-yloxy)-2H-chromen-2-one (**3g**): Off white solid, mp: 178-180 °C. IR (KBr): 2938, 1674, 1628, 1389, 1214, 1148, 751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.65 (dd, *J* =12.8 Hz, 1H), 7.52 (t, 1H), 7.34-7.21 (m, 6H), 5.78 (s, 1H), 4.60 (t, *J* =10 Hz, 1H), 2.93 (t, *J* =8.8 Hz, 2H), 2.25-2.20 (m, 1H), 1.94-1.80 (m, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.0, 160.3, 152.6, 138.1, 134.7, 132.0, 130.7, 129.4, 128.3, 127.8, 124.1, 123.3, 116.4, 116.1, 109.4, 36.5, 30.3, 29.8, 22.1 ppm. MS (ESI): *m/z* (rel. abund.%) 293 ($[M+1]^+$,100).

Pharmacological Screening

Anticoagulant activity: Anticoagulant activity of the synthesized compounds (**3a-3g**) was assessed against sodium citrate (standard) in albino rats. The blood samples were collected from albino rats by puncturing carotid artery. Compounds and sodium citrate were added to the blood separately (5 ng/ml, 10 ng/ml, 100 ng/ml). The time required for the formation of the clot was measured, and the results of anticoagulant activity of are shown in Table 2.

Compounds **3b** and **3d** have shown highest anticoagulant activity when compared to the standard sodium citrate. Compounds **3c** and **3g** have shown almost similar activity compared to the standard. Almost all the compounds were found to be more potent than standard at 5 ng/ml of concentration.

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Table 2: Anticoagulant activity of compounds (3a-g)

Compound	Clotting time		
	100 ng	10 ng	5 ng
3a	48 min	20 min	20 min
3b	135 min	100 min	92 min
3c	73 min	76 min	83 min
3d	100 min	70 min	72 min
3e	25 min	50 min	40 min
3f	33 min	35 min	43 min
3g	72min	70 min	58 min
Sodium citrate	80 min	40 min	15 min

CONCLUSIONS

C3- and *O*-alkylated 4-hydroxycoumarin derivatives were synthesized using sulfated tin oxide and screened for anti-coagulant activity. From the observation of the results of anticoagulant activity, it was concluded that, compound **3b** with -Cl group and 3d with -OCH_3 group have shown potent activity when compared to all the other compounds including standard sodium citrate. Compounds 3c and 3g have shown almost similar activity compared to the standard.

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