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Professor Josep Coll Toledano  
On the Occasion of his 70th Birthday**

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## Chemistry and Biological Activity of Coumarins at Molecular Level

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Synthetic coumarins were prepared in high yields using ionic liquids as an environmental friendly alternative. 3,4-Dimethyl-7-hydroxycoumarin (**3ab**) and 3-isopropyl-4-methyl-5,7-dihydroxycoumarin (**3bc**) showed interesting activity against *Taq* DNA polymerase with IC<sub>50</sub> values of 115.7 μM and 82.2 μM, respectively. Also, 4-methyl-7-hydroxycoumarin (**3aa**) and 4-methyl-5,7-dihydroxycoumarin (**3ba**) exhibited inhibitory activity against MMLV-RT with IC<sub>50</sub> values of 23.5 μM and 18.3 μM, respectively. These inhibitors could have importance as antiretroviral chemotherapeutic agents and also for the development of antitumor drugs.

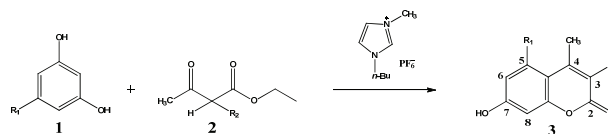
**Keywords:** Coumarins, Molecular Target, Antitumoral, Antiretroviral.

Coumarins are oxygen heterocycles widely distributed throughout the plant kingdom [1]. Coumarins such as calanolide A and inophyllums have been recognized as non-nucleoside inhibitors of HIV-1 RT. Calanolide A, isolated from *Calophyllum lanigerum* Miq. var. *austrocoriaceum* (T. C. Withmore) P. F. Stevens (Guttiferae), provided complete protection against the *in vitro* replication of HIV-1 in lymphoblastic cells [2,3]. In view of their importance as drugs, biologically active natural products and medicinally useful properties, extensive studies have been carried out on the synthesis of coumarin compounds in recent years [4-6]. A valuable method for coumarin synthesis is the Pechmann reaction using concentrated sulfuric acid as catalyst. This reaction needs a long time to obtain the products, and introduces corrosion problems. There is a strong interest to find alternative environmentally benign synthetic routes [7]. Recently, ionic liquids (ILs) have emerged as a powerful alternative to conventional organic solvents due to their particular properties [8].

Retroviruses require a reverse transcriptase (RT) to convert viral RNA into proviral DNA that can then be inserted into the host DNA. RT has become an important target for drug discovery because of its critical role in HIV production [9]. It has been reported that natural coumarins and derivatives can display anti-HIV activity through different mechanisms, including blockade of viral entry, inhibition of reverse transcriptase and interference with viral integration [10,11]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a group of small (<600 Da) hydrophobic compounds that specifically bind HIV-1 RT, acting as non-competitive inhibitors with respect to either dNTP or nucleic acid substrates [12]. Nucleoside analogues cause chain termination when they are incorporated within newly synthesized DNA, producing adverse effects and resistance problems [13]. Non-nucleoside inhibitors block RT by binding to a pocket adjacent to the catalytic site of the enzyme and thereby disrupting the conformation of several amino acids essential for a proper function. In this context, the NNRTIs have increased their importance by their specificity and low cytotoxicity [14]. Another potential target is DNA polymerases, which represent important cellular targets in the development of anticancer and antiviral agents [15,16].

As part of our continuing efforts in the search for new leads for the development of novel antiviral agents and as a continuation of a previous report [17] we are interested in the synthesis and evaluation of the antiviral activity of natural and synthetic coumarins. Herein, we describe the preparation of different semi-synthetic coumarin derivatives, and their evaluation as inhibitors of *Taq* DNA polymerase and myeloid murine leukemia virus RT (MMLV-RT). We obtained five related coumarins (Scheme 1 and Table 1) in a faster and facile method using different phenols and β-ketoesters. The advantage of using ILs focuses on high yields and short reaction times, besides being made more environmentally friendly, recyclable and high ionic strength [18].

Compounds **3aa**, **3ab**, **3ba** and **3bb** displayed similar <sup>1</sup>H NMR spectral patterns showing one or two methyl groups. Precisely, methyl groups at C-4 appeared as singlets in the region δ 2.41-2.60, while at C-3 in **3ab** and **3bb** the signals appeared at higher field at δ 2.15 and 2.08, respectively. Product **3bc** showed a doublet at δ 1.30 attributed to methyl groups at C-2' of the isopropyl group (*J* ~ 9.0 Hz) and hydrogen at C-1' resonated as a multiplet at δ 3.30. All compounds obtained showed aromatic signals at lower field (δ 6.18-7.60), attributed to the phenyl moieties.



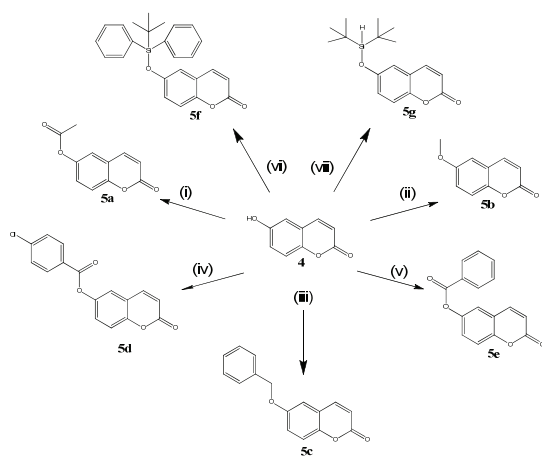
Scheme 1: 0.75 equiv POCl<sub>3</sub>, NaOH 8%, HCl 2N, 20 min, rt.

Table 1: Synthesis of coumarins **3aa-bc**.

1	R <sub>1</sub>	2	R <sub>2</sub>	Reaction Time [min]	3	Yield (%)
<b>1a</b>	H	<b>2a</b>	H	20'	<b>3aa</b>	93
<b>1a</b>	H	<b>2b</b>	CH <sub>3</sub>	20'	<b>3ab</b>	91
<b>1b</b>	OH	<b>2a</b>	H	20'	<b>3ba</b>	100
<b>1b</b>	OH	<b>2b</b>	CH <sub>3</sub>	20'	<b>3bb</b>	92
					<b>3bc</b>	73
<b>1b</b>	OH	<b>2c</b>	i-Pr	25'	<b>3ba</b>	19

Curiously, when we employed ethyl 2-isopropyl-acetoacetate, we could observe a product with the loss of an isopropyl group (**3ba**) besides the expected product **3bc** with lower yield than the reactions previously carried out. The different reactivity could possibly be due to the C-2' of the isopropyl group being a tertiary carbon, capable of forming a more stable carbocation, contrary to the above reactions. In fact, this sub-product showed a singlet signal at  $\delta$  5.85 attributed to the hydrogen atom at C-3 of the coumarin fragment and the same NMR and mass spectra as **3ba**. Coumarins **3aa**, **3ab**, **3ba** and **3bb** showed similar mass fragmentation patterns with loss of 28  $m/z$  from the molecular ion attributed to a C=O function, but **3bc** gave a different route of fragmentation. Herein, the base peak appears at  $m/z$  219, corresponding to a methyl group loss from the ion at 234  $m/z$  ( $[M]^+$ ). Then two consecutive methylene losses at 204  $m/z$  and 191  $m/z$  can occur, giving a fragment without an isopropyl side chain.

With the aim of enhancing the number of compounds for biological testing (Scheme 2) we performed chemical transformation of 6-hydroxycoumarin (**4**), which was treated with  $Ac_2O$  in the presence of pyridine, leading to the formation of compound **5a** with a typical acetyl moiety (81% yield). In the same way we used  $Me_2SO_4$  to generate a methoxy function in stoichiometric amounts (**5b**). In another set of reactions we generated different products structurally related to an extra aryl moiety, such as an *O*-benzyl **5c** (91% yield), *p*-chloro-benzoyl **5d** (83% yield) and benzoyl **5e** (41% yield) derivatives, showing new aromatic signals, and **5c** showing a singlet at  $\delta$  5.08 in the  $^1H$  NMR spectrum corresponding to an *O*-benzyl function. In order to increase lipophilicity of coumarins we carried out silylation of the hydroxyl group with TBDPSCI and DTBSCI. Some compounds with a high degree of hydrophobicity can act as NNRTIs by binding to an allosteric pocket showing, generally, low toxicity. Compound **5f** showed signals at higher field at  $\delta$  1.11, besides new aromatic signals, attributed to three methyl groups and product **5g** in the region of  $\delta$  1.06 corresponding to six methyl groups.



**Scheme 2:** (i)  $Ac_2O$ , Py, DMAP, 48 h, rt. (ii)  $Me_2SO_4$ ,  $K_2CO_3$ , acetone, 24 h, reflux. (iii) BnBr, NaH, THF, 45 min, rt. (iv) 4-Chlorobenzoyl chloride, TEA,  $Cl_2CH_2$ , 24 h, rt. (v) BzCl, TEA,  $Cl_2CH_2$ , 24 h, rt. (vi) TBDPSCI, DMAP,  $I_2$ , Py, 2 h, rt. (vii) DTBSCI, DMAP,  $I_2$ , Py, 2 h, rt.

Coumarins have been used in traditional treatments for thousands of years, and several synthetic derivatives have been prepared in the last decade. The pharmacological and biochemical properties of coumarin have been attributed to the pattern of ring substitutions [19, 20]. Taking into account this information, the family of synthetic and semi-synthetic coumarins here prepared was evaluated for biological activity by measuring the compounds ability to inhibit the reverse transcriptase of MMLV and *Taq* DNA polymerase using ddATP as reference inhibitor compound.

Assessment of the activity of *Taq* DNA polymerase was performed by the PCR technique. In an initial screening we used a concentration equal to 500  $\mu M$ ; the results revealed that analogues **3ab** and **3bc** showed inhibitory activity with  $IC_{50}$  values of  $115.7 \pm 2.3 \mu M$  and  $82.2 \pm 7.2 \mu M$ , respectively. Furthermore, we used all compounds obtained (except **3ab** and **3bc** because the RT-PCR experiment involved *Taq* DNA polymerase activity) to evaluate the reverse transcription process using also a concentration of 500  $\mu M$  for initial screening. Herein could be observed that compounds **3aa** and **3ba** produced inhibition with  $IC_{50}$  values of  $23.5 \pm 0.5 \mu M$  and  $18.3 \pm 0.3 \mu M$ , respectively. These results indicate that the obtained products using Pechmann reaction are extremely active, because two of them are able to inhibit the polymerase reaction and two others the reverse transcription event. However, neither 6-hydroxycoumarin (**4**) nor any derivative obtained by chemical transformations of **4** was shown to be active. Maybe these compounds cannot fix appropriately inside the DNA related enzymes assayed. Taking into account the structures and their hydrophobic character, it is hard to think that these coumarins act by recognizing the enzymatic active site. Probably they can be placed allosterically, for example at some hydrophobic pocket [21, 22].

In this context, while NRTIs (e.g. zidovudine, lamivudine,) act competitively at the catalytic site of the RT as DNA chain-terminating analogues of the natural deoxynucleoside triphosphates, NNRTIs (e.g. nevirapine, efavirenz) can bind to an allosteric site located about 10 Å from the catalytic site, thus leading to a noncompetitive inhibition of DNA related enzymes [23].

In summary, we have prepared a group of five coumarins using ILs as an ecological alternative in an easy way with high yields and shorter reaction times. Also, we prepared seven derivatives from 6-hydroxycoumarin. Their inhibitory activity against *Taq* DNA polymerase and MMLV-RT was evaluated, showing that **3ab/3bc** inhibited the replication process and compounds **3aa/3ba** inhibited the reverse transcription event. Thus, novel leads from these coumarins can be further developed into potential chemotherapeutic agents for antiviral or antitumor treatment.

## Experimental

**General procedures:** All the chemicals used were of analytical grade. Reactions requiring anhydrous conditions were performed under nitrogen. Dichloromethane and diethyl ether were distilled from  $CaH_2$  and Na/benzophenone, respectively, under  $N_2$  prior to use. TLC was carried out on Merck aluminum sheets coated with silica gel 60 F<sub>254</sub>. Plates were visualized by use of UV light and/or sodium permanganate 20% solution without heating. NMR spectra were measured at 500, 400 or 200 MHz ( $^1H$  NMR) and 100 or 50 MHz ( $^{13}C$  NMR) in either  $CDCl_3$  or MeOD, and chemical shifts are reported relative to internal  $Me_4Si$  ( $d = 0$ ). HRMS were obtained on a Micromass AutoSpec mass spectrometer. PCR experiments were made on a Perkin-Elmer GeneAmp 2400. *Taq* DNA polymerase and RT-MMLV used were purchased from Sigma-Aldrich, and also all the reagents for the polymerization reactions of nucleic acids.

**General procedure for coumarins synthesis using Pechmann reaction:** A mixture of either resorcinol (275 mg, 2.5 mmol) or benzene-1,3,5-triol (312 mg, 2.5 mmol) and either ethyl acetoacetate (325 mg, 2.5 mmol), ethyl 2-methyl-acetoacetate (360 mg, 2.5 mmol) or ethyl 2-isopropyl-acetoacetate (430 mg, 2.5 mmol) was carried out at room temperature in the presence of 1 mL of ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate with vigorous stirring for 15 min. Then, a catalytic amount of  $POCl_3$  (114 mg, 0.75 mmol) was added and the mixture was allowed to stir for 5 min more. Finally, NaOH 8% was added until



basic conditions were achieved, and then 2N HCl was added to neutralize the reaction, which was monitored by TLC for the disappearance of reactants. The solid formed was collected by filtration and eliminated, washing the mixture successively with ice-water and brine solution. After drying over Na<sub>2</sub>SO<sub>4</sub>, the residue was purified by Si gel CC using mixtures of *n*-hexane/EtOAc of increasing polarity. The isolated compounds were analyzed by HRMS, <sup>1</sup>H NMR, <sup>13</sup>C NMR and 2D experiments like DEPT, H,H-COSY and HETCOR. Data for synthesized coumarins are listed below.

#### 4-Methyl-7-hydroxycoumarin (3aa)

White solid.

MP: 105-107°C.

<sup>1</sup>H NMR (400 MHz, MeOD): δ 2.42 (3H, s, Me), 6.09 (1H, s, H-3), 6.69 (1H, bs, H-8), 6.82 (1H, bs, H-6), 7.57 (1H, bs, H-5).

<sup>13</sup>C NMR (100 MHz, MeOD): δ 17.28 (CH<sub>3</sub>), 102.08 (CH-8), 109.81 (CH-3), 112.41 (C), 112.94 (CH-6), 125.95 (CH-5), 154.49 (C), 155.08 (C), 161.57 (C), 162.46 (C=O).

HR-MS *m/z* (relative intensity): calculated for 176.0473 C<sub>10</sub>H<sub>8</sub>O<sub>3</sub>; found 176.0254 (96), 148.0338 (100), 147.0289 (63), 120.0346 (15), 91.0480 (21).

Yield 93 %.

#### 3,4-Dimethyl-7-hydroxycoumarin (3ab)

White solid.

MP: 186-190°C.

<sup>1</sup>H NMR (400 MHz, MeOD): δ 2.15 (3H, s, Me), 2.41 (3H, s, Me), 6.69 (1H, d, *J* = 2.4 Hz, H-8), 6.82 (1H, dd, *J* = 2.4; 8.8 Hz, H-6), 7.60 (1H, d, *J* = 8.8 Hz, H-5).

<sup>13</sup>C NMR (100 MHz, MeOD): δ 11.53 (CH<sub>3</sub>), 13.68 (CH<sub>3</sub>), 101.72 (CH-8), 112.71 (CH-6), 113.02 (C), 117.04 (C), 125.65 (CH-5), 147.93 (C), 153.43 (C), 160.29 (C), 163.19 (C=O).

HR-MS *m/z* (relative intensity): calculated for 190.0630 C<sub>11</sub>H<sub>10</sub>O<sub>3</sub>; found 190.0620 (93), 162.0665 (100), 161.0587 (78), 147.0425 (84), 91.0480 (18).

Yield 91 %.

#### 4-Methyl-5,7-dihydroxycoumarin (3ba)

Light yellow solid.

MP: 296-299°C.

<sup>1</sup>H NMR (400 MHz, MeOD): δ 2.60 (3H, s, Me), 5.85 (1H, s, H-3), 6.23 (2H, bs, H-6,8).

<sup>13</sup>C NMR (100 MHz, MeOD): δ 22.72 (CH<sub>3</sub>), 94.41 (CH-8), 98.91 (CH-6), 102.71 (C), 108.22 (CH-3), 156.67 (C), 156.89 (C), 158.18 (C), 161.56 (C), 162.64 (C=O).

HR-MS *m/z* (relative intensity): calculated for 192.0493 C<sub>10</sub>H<sub>8</sub>O<sub>4</sub>; found 191.9943 (62), 164.0463 (100), 163.0356 (32), 77.0344 (17).

Yield 100 %.

#### 3,4-Dimethyl-5,7-dihydroxycoumarin (3bb)

Yellow solid.

MP: 294-296°C.

<sup>1</sup>H NMR (400 MHz, MeOD) δ 2.08 (3H, s, Me), 2.58 (3H, s, Me), 6.19 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.0 Hz, H-6).

<sup>13</sup>C NMR (100 MHz, MeOD) δ 11.24 (CH<sub>3</sub>), 18.07 (CH<sub>3</sub>), 94.05 (CH-8), 99.13 (CH-6), 103.19 (C), 114.83 (C), 150.67 (C), 154.84 (C), 157.43 (C), 160.09 (C), 163.24 (C=O).

HR-MS *m/z* (relative intensity): calculated for 206.0579 C<sub>11</sub>H<sub>10</sub>O<sub>4</sub>; found 206.0210 (94), 178.0637 (100), 177.0560 (58), 163.0289 (66), 77.0373 (19). Yield 92 %.

#### 3-Isopropyl-4-methyl-5,7-dihydroxycoumarin (3bc)

White solid.

MP: 262-264°C.

<sup>1</sup>H NMR (400 MHz, MeOD): δ 1.30 (6H, d, *J* = 9.0 Hz, 2Me), 2.65 (3H, s, Me), 3.30 (1H, m, CH), 6.18 (1H, d, *J* = 2.0 Hz, H-8), 6.23 (1H, d, *J* = 2.0 Hz, H-6).

<sup>13</sup>C NMR (100 MHz, MeOD): δ 17.52 (CH<sub>3</sub>), 18.82 (2CH<sub>3</sub>), 27.37 (CH-2'-*i*-Pr), 94.15 (CH-8), 99.16 (CH-6), 103.37 (C), 124.20 (C), 149.96 (C), 155.04 (C), 157.66 (C), 160.07 (C), 161.16 (C=O).

HR-MS *m/z* (relative intensity): calculated for 234.0892 C<sub>13</sub>H<sub>14</sub>O<sub>4</sub>; found 234.0877 (43), 219.0619 (100), 204.2907 (22), 191.0351 (48), 77.0355 (11).

Yield 73 %.

**6-Acetylcoumarin (5a):** A solution of 6-hydroxycoumarin (150 mg, 0.93 mmol), and DMAP (80 mg, 0.66 mmol) in pyridine (1 mL) and Ac<sub>2</sub>O (1 mL) was stirred at room temperature for 48 h. Then the mixture was poured into cold water and Et<sub>2</sub>O and washed with HCl (10%) and NaHCO<sub>3</sub> (10%). The organic phase was separated, dried (MgSO<sub>4</sub>), filtered, and purified by CC using mixtures of *n*-hexane/EtOAc in increasing polarities, to afford a yellow solid.

Yield 81%.

MP: 119-124°C.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 2.33 (3H, s, Ac), 6.46 (1H, d, *J* = 9.6 Hz, H-3), 7.28 (3H, bs, H-5, 7, 8), 7.66 (1H, d, *J* = 9.6 Hz, H-4).

**6-Metoxycoumarin (5b):** A solution of 6-hydroxycoumarin (100 mg, 0.62 mmol), K<sub>2</sub>CO<sub>3</sub> (150 mg, 1.10 mmol) and Me<sub>2</sub>SO<sub>4</sub> (151 mg, 1.2 mmol) in acetone (40 mL) was stirred at 65°C for 24 h. The mixture was poured into cold water and Et<sub>2</sub>O and washed with HCl (10%) and NaHCO<sub>3</sub> (10%). The organic phase was separated, dried (MgSO<sub>4</sub>), filtered, and purified by CC using mixtures of *n*-hexane/EtOAc in increasing polarities, to afford a yellow solid.

Yield 100 %.

MP: 116-120°C.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 3.85 (3H, s, -OMe), 6.43 (1H, d, *J* = 9.6 Hz, H-3), 6.91 (1H, d, *J* = 2.8 Hz, H-5), 7.18 (1H, dd, *J* = 2.8; 9.0 Hz, H-7) 7.65 (1H, d, *J* = 9.6 Hz, H-4) (1H, d, *J* = 9.0 Hz, H-8).

**6-Benzoyloxycoumarin (5c):** A solution of 6-hydroxycoumarin (100 mg, 0.62 mmol), NaH (24 mg, 1.00 mmol) and BnBr (170 mg, 1.00 mmol) in THF (25 mL) was stirred at room temperature for 45 min. Then *tetra*-butylammonium fluoride (261 mg, 1.00 mmol) was added to quench the reaction. The mixture was poured into cold water and Et<sub>2</sub>O. The organic phase was separated, dried (MgSO<sub>4</sub>), filtered, and purified by CC using mixtures of *n*-hexane/EtOAc in increasing polarities, to afford a light yellow solid.

Yield 91 %.

MP: 146-150°C.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 5.08 (2H, s, -OBn), 6.38 (1H, d, *J* = 9.6 Hz, H-3), 6.97-7.50 (8H, aromatic), 7.61 (1H, d, *J* = 9.6 Hz, H-4)

**6-(*p*-Chlorobenzoyloxy)-coumarin (5d):** A solution of 6-hydroxycoumarin (100 mg, 0.62 mmol), Et<sub>3</sub>N (133 mg, 1.30 mmol) and *p*-chlorobenzoyl chloride (226 mg, 1.30 mmol) in Cl<sub>2</sub>CH<sub>2</sub> (30 mL) was stirred at room temperature for 24 h. The mixture was poured into cold water and CHCl<sub>3</sub>. The organic phase was separated, dried (MgSO<sub>4</sub>), filtered, and purified by CC using mixtures of *n*-hexane/EtOAc in increasing polarities, to afford a white solid.

Yield 41 %.

MP: 158-160°C.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 6.49 (1H, d, *J* = 9.6 Hz, H-3), 7.39 (3H, bs, H-5, 7, 8), 7.53 (2H, dd, *J* = 2.0; 6.8 Hz, H-14<sub>a-b</sub>) 7.69 (1H, d, *J* = 9.6 Hz, H-4), 8.14 (2H, dd, *J* = 2.0; 6.8 Hz, H-13<sub>a-b</sub>).

**6-Benzoyloxy-coumarin (5e):** The synthesis of this compound was made in an analogous manner to that reported for compound 5d,

using BzCl (182 mg, 1.30 mmol) instead of *p*-chlorobenzoyl chloride, to afford a white solid.

Yield 83 %.

MP: 190-193°C.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 6.48 (1H, d, *J*= 9.4 Hz, H-3), 7.40-7.65 (6H, aromatic), 7.69 (1H, d, *J*= 9.4 Hz, H-4), 8.21 (2H, dd, *J*= 2.0; 7.0 Hz, H-13<sub>a-b</sub>).

**6-(*t*-Butyldiphenylsilyloxy)-coumarin (5f):** A solution of 6-hydroxycoumarin (200 mg, 1.23 mmol), DMAP (150 mg, 1.23 mmol), sublimated I<sub>2</sub> (127 mg, 0.5 mmol), TBDPSCI (358 mg, 1.30 mmol) in pyridine (5 mL) was stirred at room temperature for 2 h. The mixture was poured into cold water and Et<sub>2</sub>O and washed with HCl (10%) and NaHCO<sub>3</sub> (10%). The organic phase was separated, dried (MgSO<sub>4</sub>), filtered, and purified by CC using mixtures of *n*-hexane/EtOAc in increasing polarities, to afford a white solid.

MP: 93-94°C.

Yield 61 %.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.11 (9H, s, *t*-Bu), 6.31 (1H d, *J*= 9.6 Hz, H-3), 6.79-7.72 (14H, aromatic).

**6-(*Di-t*-butylsilyloxy)-coumarin (5g):** The synthesis of this compound was made in an analogous manner to that reported for compound **5f**, using DTBSCI (231 mg, 1.30 mmol) instead of TBDPSCI, to afford a yellow oil.

Yield 68 %.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.06 (18H, s, 2*t*-Bu), 4.45 (1H, s, H-Si), 6.41 (1H, d, *J*= 9.6 Hz, H-3), 6.98-7.22 (3H, bs, H5, 7, 8), 7.62 (1H, d, *J*= 9.6 Hz, H-4).

**Molecular experiments:** Methodology for PCR, RT-PCR, and analysis of PCR products has been described previously in ref. [16] and Supplementary Material.

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## References

- Estéves-Braun A, González A. (1997) Coumarins. *Natural Product Reports*, **14**, 465-475.
- Kashman Y, Gustafson K, Fuller R, Cardellina J, Mc Mahon J, Currens M, Buckheit R, Hughes S, Cragg G, Boyd M. (1992) HIV inhibitory natural products. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. *Journal of Medicinal Chemistry*, **35**, 2735-2743.
- Patil A, Freyer A, Eggleston D, Haltiwanger R, Bean M, Taylor P, Caranfa M, Breen A, Bartus H, Johnson R, Hertzberg R, Westley J. (1993) The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, *Calophyllum inophyllum* Linn. *Journal of Medicinal Chemistry*, **36**, 4131-4138.
- Xin-Hua L, Hui-Feng L, Jin C, Yang Y, Bao-An S, Lin-Shan B, Jing-Xin L, Hai-Liang Z, Xing-Bao Q. (2010) Synthesis and molecular docking study of novel coumarin derivatives containing 4,5-dihydropyrazole moiety as potential antitumor agents. *Bioorganic and Medicinal Chemistry Letters*, **20**, 5705-5708.
- Nader G. (2012) Synthesis of coumarins via Pechmann reaction catalyzed by 3-methyl-1-sulfonic acid imidazolium hydrogen sulfate as an efficient, halogen-free and reusable acidic ionic liquid. *Catalysis Science Technology*, **2**, 1633-1636.
- Temitope O, Olomola R, Kevin A, Lobb Y, Perry T. (2010) Towards the synthesis of coumarin derivatives as potential dual-action HIV-1 protease and reverse transcriptase inhibitors. *Tetrahedron Letters*, **51**, 6325-6328.
- Rajitha B, Naveen P, Someshwara J, Venu P, Narsimha R, Thirupathi R. (2006) Dipyridine copper chloride catalyzed coumarin synthesis via Pechmann condensation under conventional heating and microwave irradiation. *Arkivoc*, **xii**, 23-27.
- Dong F, Jian C, Kai G, Qun-Rong S, Xin-Li Z, Zu-Liang L. (2008) A green and novel procedure for the preparation of ionic liquid. *Journal of Fluorine Chemistry*, **129**, 108-111.
- Otsuka J, Kikuchi N, Kojima S. (1999) Similarity relations of DNA and RNA polymerases investigated by the principal component analysis of amino acid sequences. *Biochimica et Biophysica Acta*, **221**, 1434-1443.
- Bedoya L, Beltrán M, Sancho R, Olmedo D, Sánchez-Palomino S, Olmo E, López-Pérez J, Muñoz E, San Feliciano A, Alcamí J. (2005) 4-phenylcoumarins as HIV transcription inhibitors. *Bioorganic and Medicinal Chemistry Letters*, **15**, 4447-4450.
- Saiz-Urra L, Pérez-González M, Fall Y, Gómez G. (2007) Quantitative structure-activity relationship studies of HIV-1 integrase inhibition. *European Journal of Medicinal Chemistry*, **42**, 64-70.
- Sluis-Cremer N, Tachedjian G. (2008) Mutagenesis identifies the critical regions and amino acid residues of suid herpesvirus DNA-binding protein required for DNA binding and strand invasion. *Virus Research*, **134**, 147-156.
- Jin-Soo L, Hyung-Soo S, Yong-Kyun P, Sang-Jin P, Joon-Su S, Wang-Yong Y, Hak-Dong L, Whui-Jung P, Yong-Ho C. and Sang-Wook, L. (2002) 2,5-Pyridinedicarboxylic acid derivatives as non-nucleosidic reverse transcriptase inhibitors of hepatitis B virus. *Bioorganic and Medicinal Chemistry Letters*, **12**, 2715-2717.
- Zahouily M, Rakik J, Lazar M, Bahlaoui M, Rayadh A, Komih N. (2007) Exploring QSAR of non-nucleoside reverse transcriptase inhibitors by artificial neural networks: HEPT derivatives. *Arkivoc*, **xiv**, 245-256.
- Pungitore C, Leon L, García C, Martín V, Tonn C, Padrón J. (2007) Novel antiproliferative analogs of the *Taq* DNA polymerase inhibitor catalpol. *Bioorganic and Medicinal Chemistry Letters*, **17**, 1332-1335.
- De Clercq E. (1995) Toward improved anti-HIV chemotherapy: Therapeutic strategies for intervention with HIV infection. *Journal of Medicinal Chemistry*, **38**, 2491-2517.
- Garro H, Manzur J, Ciuffo G, Tonn C, Pungitore C. (2014) Inhibition of reverse transcriptase and *Taq* DNA polymerase by compounds possessing the coumarin framework. *Bioorganic and Medicinal Chemistry Letters*, **24**, 760-764.
- Keskin S, Kayrak-Talay D, Akman U, Hortacsu O. (2007) Ionic liquids towards supercritical fluid applications. *Supercritical Fluids*, **43**, 150-180.
- Béthune M. (2010) Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989-2009). *Antiviral Research*, **85**, 75-90.
- Wu L, Wang X, Xu W, Farzaneh F, Xu R. (2009) The structure and pharmacological functions of coumarins and their derivatives. *Current Medicinal Chemistry*, **16**, 4236-4260.
- Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. (2005) Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. *Current Medicinal Chemistry*, **12**, 887-916.
- Silva F, De-Simone S. (2004) S1 sub-site in snake venom thrombin-like enzymes: can S1 sub-site lipophilicity be used to sort binding affinities of trypsin-like enzymes to small-molecule inhibitors? *Bioorganic and Medicinal Chemistry*, **12**, 2571-2587.
- De Clercq E. (2004) Non-nucleoside reverse transcriptase inhibitors (NNRTIs): past, present, and future. *Chemistry & Biodiversity*, **1**, 44-64.

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# Natural Product Communications

## 2014

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