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Synthesis and antitumor activity of 4-hydroxycoumarin derivatives

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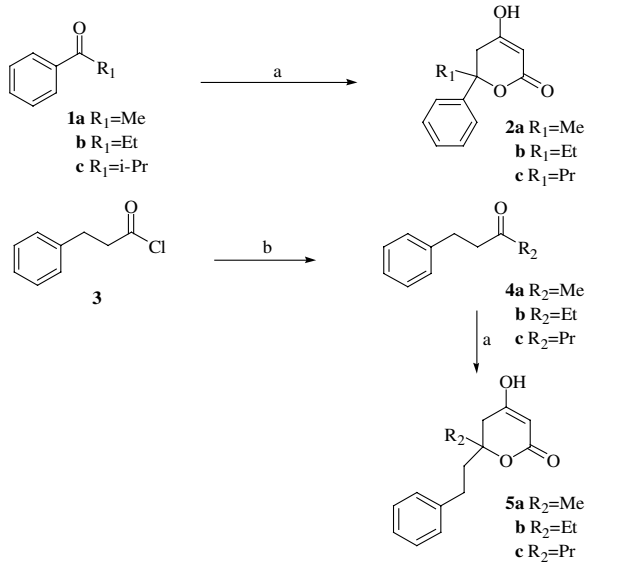
Abstract—A series of 4-hydroxycoumarin derivatives was prepared and evaluated for antitumor activity. The key fragments were **2a–c**, **5c**, **12b**, **13b**, **17**, and **18** which were prepared via dianion ring cyclization, Friedel–Crafts acylation, and Reformatsky reaction. Compound **20b** showed the most potent antitumor activity among the total 12 derivatives and compounds **19a** and **19b** exhibited efficacy comparable to etoposide in vitro antitumor activity.

Coumarin and some of its hydroxylated derivatives containing a benzene ring fused with a γ-pyrone ring possess important chemical reactivity[1] and are naturally occurring compounds, which exhibit antibacterial,[2] antioxidant,[3] anticancer,[4] and antiallergy activities.[5] They are frequently used as intermediates in the production of dyes[6] and herbicides.[7] Derivatives of 4-hydroxycoumarin have been used successfully as potent warfarin-type anticoagulents (i.e., bromadiolone,[8] brodifacoum,[9] flocoumafen,[10] thioflocoumafen[11]) with low toxicity. As compared to the first-generation multidose warfarin-type anticoagulants, these compounds are more potent in rodents and require reduced feeding periods and baits.[12] Flocoumafen is the most effective anticoagulant agent of the 4-hydroxycoumarin derivatives. Moreover, its sulfur analog, thioflocoumafen showed improved potency anticoagulant activity than flocoumafen with lower toxicity.[11] Recent reports describe that 4-hydroxycoumarin derivatives exhibit biological activities as anticoagulant,[13] and nonpeptide human immunodeficiency virus (HIV) protease inhibitors.[14]

In a preliminary communication, we have reported[15] the efficient synthesis of 4-hydroxycoumarin and its analogs. Herein, we describe the versatile synthetic routes and antitumor activities of 4-hydroxycoumarin derivatives.

The syntheses of 4-hydroxy-5,6-dihydropyrones (**2a–c**, **5c**) are shown in Scheme 1. We have used commercially available alkylphenones (**1a–c**), which were reacted with ethyl acetoacetate using sodium hydride and then *n*-butyllithium. To the dianion of ethyl acetoacetate was added alkylphenones (**1a–c**) and they were subsequently



Scheme 1. Reagents and conditions: (a) (i) $CH_3COCH_2CO_2Et$, NaH, *n*-BuLi/THF, 0 °C, 1 h; (ii) 0.1 N NaOH/THF, rt 3 h (two steps; for **2a**: 65%; for **2b**: 61%, for **2c**: 64% and for **5c**: 48%); (b) $CH_3CH_2CH_2Br$, Mg, $CdCl_2$/THF, reflux, 4 h (60%).

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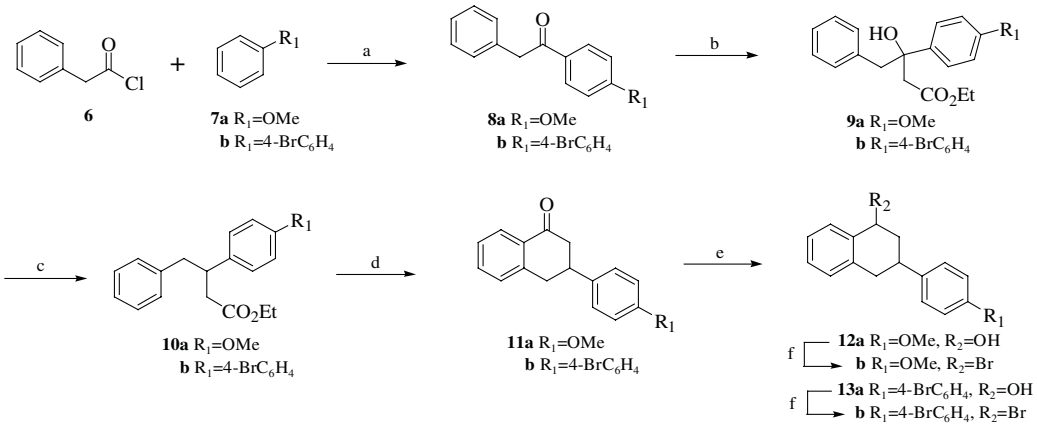
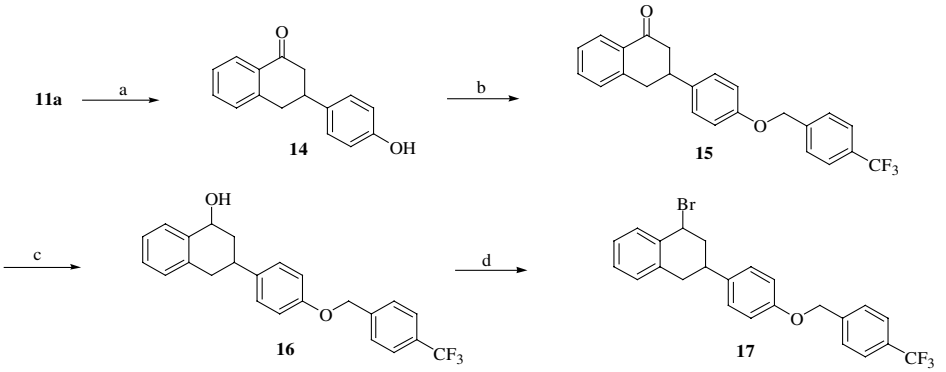
treated with 0.1 N NaOH (aq) to afford the 4-hydroxy-5,6-dihydropyrones (**2a**–**c**).[16] The condensation of hydrocinnamoyl chloride with propylmagnesium bromide in the presence of cadmium chloride directly afforded 1-phenyl-3-hexanone (**4c**) in 48% yield, which was reacted with the dianion of ethyl acetoacetate, followed by hydrolysis of the intermediate β-keto ester and spontaneous ring cyclization upon acidification to give 6-phenethyl-6-propyl dihydropyrone (**5c**).[17] In an effort to prepare 6-phenethyl-6-methyl dihydropyrone (**5a**) and 6-phenethyl-6-ethyl dihydropyrone (**5b**), several basic reaction conditions were attempted using 4-phenyl-2-butanone (**4a**) and 1-phenyl-3-pentanone (**4b**) as starting materials. Unfortunately, these reactions failed to afford desired products, giving decomposed products. The acylation of anisole (**7a**) or 4-bromobiphenyl (**7b**) with phenylacetyl chloride (**6**) to directly afford ketones **8a** and **8b**. Reformatsky reaction with ethyl bromoacetate gave 3-hydroxy esters **9a** and **9b** in high yields. Subsequent dehydroxylation of ethyl esters **9a** and **9b** was accomplished with triethylsilane and boron trifluoride.[18] The resulting ethyl esters **10a** and **10b** were hydrolyzed under basic conditions and cyclized using polyphosphoric acid to give 3-(4-methoxyphenyl)-1-tetralone (**11a**) and 3-(*p*-bromobiphenyl-4-yl)-1,2,3,4-tetrahydronaphthalen-1-one (**11b**). Reduction of **11a** and **11b** with sodium borohydride stereospecifically afforded *cis*-3-(4-methoxyphenyl)-1-tetralol (**12a**) and 3-(*p*-bromobiphenyl-4-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (**12b**) in high yields (Scheme 2). The apparently exclusive formation of *cis* isomer is somewhat surprising in this instance, since no particular steric hindrance to the approach of the reducing species from either side would be anticipated from molecular models. In *cis*-3-(4-methoxyphenyl)-1-tetralol (**12a**), the alicyclic ring assumes a half chair conformation with the C-3 substituent occupying an equatorial position based on ^{1}H NMR study.[19] Subsequent in situ bromination with PBr_3 of alcohols **12a** and **13a** afforded **12b** and **13b** in good yields.[20] Cleavage of the methyl ether of **11a** was accomplished with hydrobromic acid in acetic acid to give **14** in 93% yield and the resulting phenol was O-alkylated with 3-(trifluoromethyl)benzyl bromide, sodium hydride/THF to give **15** in 67% yield. Ketone **15** was reduced to the corresponding alcohol **16** with sodium borohydride/methanol. Treatment of **16** with phosphorus tribromide gave bromide **17** (44%, two steps) as an unstable intermediate (Scheme 3).



Scheme 2. Reagents and conditions: (a) $AlCl_3/CH_2Cl_2$, −10 °C, 16 h (89%, 88%); (b) Zn, I_2, $BrCH_2CO_2Et$/benzene, reflux, 1 h (97%, 95%); (c) $(Et)_3SiH$, TFA, BF_3EtO_2/CH_2Cl_2, reflux, 8 h (90%, 85%); (d) (i) KOH/H_2O, reflux, 8 h (92%, 93%); (ii) PPA, 80 °C, 1 h (85%, 80%); (e) $NaBH_4$/EtOH, rt, 2 h (87%, 85%); (f) PBr_3/CH_2Cl_2, −10 °C 1 h.

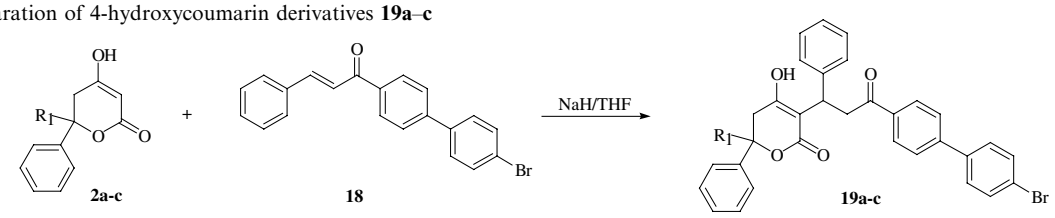


Scheme 3. Reagents and conditions: (a) HBr/AcOH, reflux, 6 h (93%); (b) 3-(trifluoromethyl)benzyl bromide, NaH/THF, 0 °C, 1 h (67%); (c) $NaBH_4$/MeOH, rt, 2 h (87%); (d) PBr_3/CH_2Cl_2, −10 °C, 1 h (50%).

The coupling reaction of 4-hydroxy-5,6-dihydroxypyrones **2a–c** and freshly prepared α,β-unsaturated ketone **18**[21] was accomplished through 1,4-Michael addition to give 4-hydroxycoumarin derivatives **19a–c**[22] in good yields (Table 1). The condensation of 4-hydroxycoumarins **2a** and **2b**, **5c** and tetrahydronaphthyl bromides **12b**, **13b**, **17** gave the alkylated products **20a–i**[22] (Table 2). The diastereomeric ratios and yields are summarized in Table 2. 4-Hydroxycoumarin derivatives **19a–c**, **20a–i** were obtained as diastereomeric mixtures. Unfortunately, these diastereomeric mixtures could not be separated by flash column chromatography using silica gel, florisil or neutral alumina. The diastereomeric ratios of compounds **20a–i** were determined by normal phase HPLC analysis (40% ethyl acetate in *n*-hexanes, PDA 254.0 nm, Table 2). However, the diastereomeric ratios of **19a–c** remains undetermined.

The in vitro cytotoxicities of these 4-hydroxycoumarin derivatives **19a–c**, **20a–i** were evaluated in five human tumor cell lines, A549 (nonsmall cell lung carcinoma), SK-OV-3 (ovarian carcinoma), SK-MEL-2 (melanoma), XF498 (CNS carcinoma) and HCT-15 (colon carcinoma) by the SRB (sulforhodamine B) method and the results are summarized in Table 3. Compounds **19a**, **19b** and **20b** showed cytotoxic activity. Compound

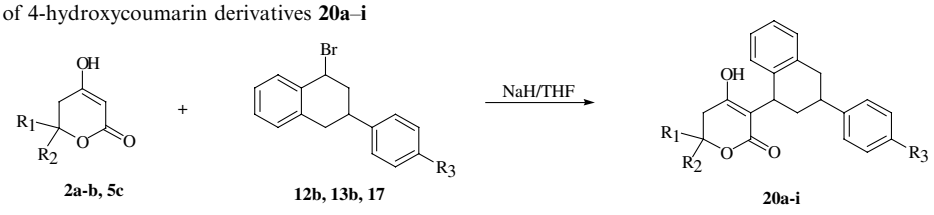
Table 1. Preparation of 4-hydroxycoumarin derivatives **19a–c**

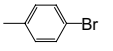
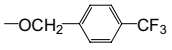
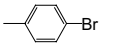
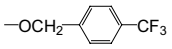
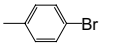
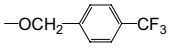


Entry	R_1	Product	Yield (%)[a]
1	Me	**19a**	70
2	Et	**19b**	75
3	Pr	**19c**	72

[a] Isolated yields.

Table 2. Preparation of 4-hydroxycoumarin derivatives **20a–i**



Entry	R_1	R_2	R_3	Product	Selectivity (dr)[a]	Yield (%)[b]
1			OMe	**20a**	2.2:1	68
2	Me	Ph		**20b**	1.9:1	61
3				**20c**	1.7:1	70
4			OMe	**20d**	2.0:1	65
5	Et	Ph		**20e**	1.7:1	71
6				**20f**	2.0:1	66
7			OMe	**20g**	2.1:1	69
8	Pr	$-(CH_2)_2Ph$		**20h**	1.7:1	72
9				**20i**	1.8:1	75

[a] dr = diastereoselective ratio; diastereoselectivity was determined based on HPLC analysis.
[b] Isolated yields.

Table 3. In vitro antitumor activity of 4-hydroxycoumarin derivatives **19a–c** and **20a–i**

Compounds	IC_{50} (μM)[a]				
	A549[b]	SK-OV-3	SK-MEL-2	XF-498	HCT-15
19a	9.18	5.69	8.54	6.73	8.59
19b	8.13	5.74	8.51	6.59	6.71
20b	1.83	1.80	2.05	1.36	1.31
19c,**20a**,**20c–i**	>10	>10	>10	>10	>10
Etoposide[c]	1.06	3.46	4.13	3.14	1.45

[a] IC_{50}: concentration which produces 50% inhibition of proliferation after 72 h of incubation.
[b] Cell lines: A549: human lung tumor, SK-OV-3: human ovarian tumor, SK-MEL-2: human melanoma tumor, XF 498: human brain tumor, HCT-15: human colon tumor.
[c] Etoposide: compared material.

20b is superior to etoposide against human tumor cell lines tested. The IC_{50} values of compound **20b** were 1.83, 1.80, 2.05, and 1.36 μM against the SK-OV-3, SK-MEL-2, and XF498, whereas those of etoposide were 3.46, 4.13, and 3.14 μM. When the O-trifluorobenzyl group was used as substituent at C-3 (compounds **20c**, **20f**, and **20i** in Table 2), the resulting compounds showed significantly reduced antitumor activity. Interestingly, the compounds with the relatively small C-6 alkyl substituent (**19a** and **20b**) exhibited similar or higher in vitro cytotoxicities when compared to compounds where one of the substituent was a large alkyl side chain (compounds **19b–c** and **20e**).

Measurement of antitumor activity: Human cancer cell lines of the lung (A549), ovarian (SK-OV-3), melanoma (SK-MEL-2), brain (XF498), and colon (HCT15) were used for cytotoxicity test in vitro using the SRB (sulforhodamine B) assay.[23] They were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cell cultures were passaged once or twice weekly by using trypsin-EDTA to detach the cells from their culture flasks. The rapidly growing cells were harvested, counted, and incubated at the appropriate concentration ($1–2 \times 10^4$ cells/well) in 96-well plates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate and incubated for 48 h at 37 °C under 5% CO_2/95% air atmosphere in a humidified incubator. The culture cells were fixed with 10% cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound stain with 10 mM of unbuffered Trisma base solution (pH 10.5) using gyratory shaker, the absorbance at 520 nm was measured spectrophotometrically in a microplate reader. Cytotoxic activity was evaluated by measuring the concentration of a compound, which was required to inhibit the protein synthesis by 50% (IC_{50}) and compared with that of etoposide.

In conclusion, a simple preparation of 4-hydroxycoumarin derivatives has been described. The synthetic strategies involve the use of well-known Friedel–Crafts acylation, Reformatsky reaction, and 1,4-Michael addition. Compounds **19a** and **19b** moderate activity in the five cell lines tested. We have found that the compound **20b** exhibited the most potency with IC_{50} values ranging from 1.31 to 2.05 μM.

Acknowledgements

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22. General procedure for the preparation of 4-hydroxycoumarin derivatives **19a–c** and **20a–i**: To a stirred solution of 4-hydroxy-5,6-dihydropyrones (**2a–c**, **5c**, 0.46 mmol) in dry THF (10 mL) was added NaH (60%, washed with *n*-hexanes, 0.69 mmol) at 0 °C, and the mixture was stirred at that temperature for 30 min. A solution of bromides **12b**, **13b**, and **17–18** (1.0 mmol) in dry THF (10 mL) was added dropwise at 0 °C, and the mixture was warmed to room temperature for 20 min. The resulting mixture was refluxed for 2 h. The mixture was quenched by addition of ice water (20 mL), and the solvent was removed under reduced pressure. The residue was treated with dichloromethane (20 mL). The aqueous phase was acidified with 2 N HCl to pH 2–3 at 0 °C, and extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give 4-hydroxycoumarin derivatives, which were purified by flash column chromatography (silica gel, ethyl acetate:*n*-hexanes, 3:1) to give **19a–c** and **20a–i**. Selected data for**19a**: mp 215–216 °C; IR (neat, NaCl) 3566, 2925, 2855, 1683, 1668, 1448, 1386, 1075, 815 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.61 (br s, 1H), 8.16 (d, J = 8.5 Hz, 2H), 7.72–7.04 (m, 14H), 6.82 (d, J = 7.5 Hz, 2H), 4.51 (dd, J = 11.0, 11.0 Hz, 1H), 4.41 (d, J = 10.5 Hz, 1H), 3.63 (d, J = 12.0 Hz, 1H), 3.17 (d, J = 18.0 Hz, 1H), 2.98 (d, J = 18.0 Hz, 1H), 1.62 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.1, 166.3, 164.0, 145.5, 143.5, 140.4, 138.3, 134.8, 132.1, 129.3, 128.8, 128.5, 127.8, 127.6, 127.2, 127.1, 125.8, 124.4, 122.8, 108.1, 80.3, 40.9, 39.0, 31.4, 30.1; HRMS calcd. for $C_{33}H_{28}BrO_4$: 567.1171 $[M+H]^+$, found: 567.3391; **19b**: mp 212–213 °C; IR (neat, NaCl) 3649, 2928, 2859, 1684, 1665, 1448, 1387, 1270, 1075, 814 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.59 (br s, 1H), 8.16 (d, J = 8.0 Hz, 2H), 7.72–7.03 (m, 14H), 6.80 (d, J = 7.5 Hz, 2H), 4.50 (dd, J = 11.0, 10.5 Hz, 1H), 4.39 (d, J = 10.5 Hz, 1H), 3.62 (d, J = 18.5 Hz, 1H), 3.11 (d, J = 18.0 Hz, 1H), 2.96 (d, J = 17.5 Hz, 1H), 1.90 (q, J = 7.5 Hz, 2H), 0.79 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.1, 166.2, 164.1, 145.4, 142.0, 140.4, 138.3, 134.9, 132.1, 129.3, 128.8, 128.3, 127.8, 127.6, 127.1, 127.0, 125.7, 125.1, 122.9, 108.2, 80.1, 40.9, 37.0, 36.3, 34.1, 8.3; HRMS calcd. for $C_{34}H_{30}BrO_4$: 581.1327 $[M+H]^+$, found: 581.0770.
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