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Note

Synthesis of monomethylated dimeric benzopyrans as HIV-1 and HIV-2 inhibitors: Part I

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Mono methylated dimeric coumarins have been synthesized by condensation of 6- or 8-methyl-4-hydroxycoumarins with different aromatic aldehydes. Twenty three compounds of the two series have been screened for anti-HIV activity against HIV-1 and HIV-2 strains.

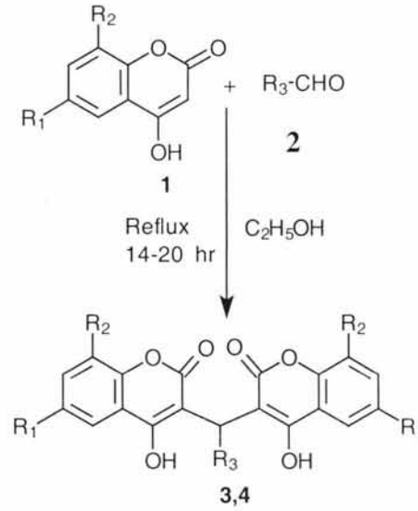
Warfarin and its analogs have been identified for structure based design of non peptidic HIV protease inhibitors and 4-hydroxycoumarin was identified as a lead structure[1]. The calonolides bearing coumarin features have also been modified for anti-HIV activity[2]. The 4-hydroxypyrone ring was suggested as a pharmacophore based on its crystal structure[3]. The sulfonamide containing 4-hydroxycoumarins and 4-hydroxy-2-pyrones were studied as protease inhibitory template[4]. Simultaneously number of dimeric and tetrameric coumarins were explored for their inhibitory potency for another enzyme responsible for HIV i.e. integrase. Many structural modifications explained that two aryl units having a central linker should show high inhibitory potency. The activity of "horizontal" and "vertical" dimer were compared and studied in detail. The unsubstituted 4-hydroxycoumarin and 4,7-dihydroxycoumarin were used for formation of dimeric coumarins, and also similar series of compounds to study inhibition of HIV-1 for both enzymes integrase and protease[5-7].

As the alkylated 4-hydroxycoumarins have not been studied earlier, this has prompted us to undertake synthesis of monomethylated coumarin derivatives at different position in the benzenoid part. In the present study, synthesis of 6-methyl-4-hydroxycoumarin and 8-methyl-4-hydroxycoumarin were carried out and converted into respective dimers by using various aromatic aldehydes for evaluation of their anti HIV-1 and HIV-2 activities.

The compounds were screened for their HIV-1 and HIV-2 inhibitory activities as per reported method[8], using strains HIV-1 (IIIB) and HIV-2 (ROD). The EC50, CC50 and EC90 values were determined of newly synthesized 23 compounds. During study of both series of compounds **3b-k** and **4b-j** (**Scheme I, Table I**), it was found in primary findings that the central aryl linker showed some effect on the anti-HIV activity. However, introduction of methyl group either at 6th or 8th position doesn't seem to improve the potency. This may suggest that though such compounds are identified and reported as both protease and integrase inhibitors, monomethyl congeners show poor HIV-1 and HIV-2 activity as the selectivity index (SI) in all cases is less than one. Further work on modification of structure is on anvil and the findings will be reported soon. Antiviral activity data are given in **Table II**.

Experimental Section

General. Melting points were determined by an open capillary method and are uncorrected. IR(KBr)



3a-k : $R_1 = CH_3$, $R_2 = H$
R_2 = aryl aldehydes

4a-i : $R_1 = H$, $R_2 = CH_3$
R_3 = aryl aldehydes

Scheme I

Table I—Physical data of monomethylated dimeric coumarins

Compd	Substitution			Reaction period	Yield	m.p.
	R_1	R_2	R_3	(hr)	(%)	°C
3a	CH_3	H	H	14	60	270
3b	CH_3	H	2-OCH_3-C_6H_4	16	65	217-18
3c	CH_3	H	4-OCH_3-C_6H_4	16	62	210-12
3d	CH_3	H	2-Cl-C_6H_4	18	60	253-55
3e	CH_3	H	2,4-$(Cl)_2$-C_6H_3	15	65	230-32
3f	CH_3	H	2-NO_2-C_6H_4	20	60	212-13
3g	CH_3	H	3-NO_2-C_6H_4	20	62	266-68
3h	CH_3	H	4-OH-C_6H_4	12	63	230-31
3i	CH_3	H	4-*N*,*N*$(CH_3)_2$-N-C_6H_4	14	60	219-20
3j	CH_3	H	3-OCH_3,4-OH-C_6H_3	18	65	194-96
3k	CH_3	H	3,4,5-$(OCH_3)_3$-C_6H_2	17	60	219-20
4a	H	CH_3	H	24	60	283
4b	H	CH_3	2-OCH_3-C_6H_4	15	60	198-200
4c	H	CH_3	4-OCH_3-C_6H_4	15	55	267-68
4d	H	CH_3	2-Cl-C_6H_4	17	57	178-80
4e	H	CH_3	4-Cl-C_6H_4	17	61	290-92
4f	H	CH_3	2,4-$(Cl)_2$-C_6H_3	18	58	203-05
4g	H	CH_3	4-SCH_3-C_6H_4	20	62	260-62
4h	H	CH_3	3-Br-C_6H_4	21	65	230-52
4i	H	CH_3	3-OCH_3,4-OH-C_6H_3	20	56	250-52
4j	H	CH_3	4-*N*,*N*$(CH_3)_2$-N-C_6H_4	14	63	220-22
4k	H	CH_3	4-NO_2-C_6H_4	16	65	252
4l	H	CH_3	3-Cl-C_6H_4	19	75	200-03

spectra were recorded on a Shimadzu IR-435 spectrophotometer (υmax in cm^{-1}). ^1H NMRspectra were recorded in $CDCl_3$ and DMSO-d_6 on a 300 MHz Bruker FT-NMR spectrophotometer using TMS as internal standard. The purity of the compounds was monitored and checked by TLC using silica gel - G during reaction.

General procedure for the preparation of 4-hydroxycoumarin 1. It was prepared according to the method of Shah and coworkers[9-10].

Preparation of 3,3'-(2-chorobenzylidene)-bis-(6-methyl-4-hydroxycoumarin)[11-12] **3d.** 6-Methyl-4-hydroxycoumarin (0.02 *M*, 3.52g) was dissolved in 30 mL of ethanol and heated on a water-bath, just to get clear solution. 2-Chlorobenzaldehyde (0.01 *M*, 1.30 g) was added to the hot solution and refluxed for 18 hr. The solvent was distilled off and 3,3'-(2-chloro benzylidene) bis (6-methyl-4-hydroxycoumarin) seperated out as crystals. It was recrystallized from ethanol as colourless crystals. m.p. 253-55°C (Yield 59%); IR (KBr): 1661 (coumarin, >C=O), 1228 (C-O-C), 710 cm^{-1} (C-Cl); ^1H NMR (300 MHz, $CDCl_3$); 6.21 (s, C_3-H), 2.39 (s, C_6-2×CH_3), 7.19 (d, *J*=8.4 Hz, C_7-H), 7.27(d, *J*=5.4 Hz, C_5-H), 7.30(d, *J*=5.7 Hz), 7.42 (d, *J*=1.2 Hz, C_8-H); 7.45(d, *J*=1.8 Hz, Ar-H)); MS (EI) : m/z 474.5 (M^+, 1.8%), 364 (M-Cl.C_6H_4, 16.2%), 446 (M-2×CH_3, 60.3 %). Anal. Calcd. for $C_{27}H_{19}ClO_6$: C, 68.28; H, 4.00; Cl, 7.48. Found : C, 68.30; H, 3.97; Cl, 7.51%.

3,3'-(3-Methoxy-4-hydroxybenzylidine)-bis(6-methyl-4-hydroxycoumarin) 3j: IR (KBr) : 3501 (OH), 1658 (>C=O, coumarin), 1345 (OH, b), 1223 cm^{-1}(C-O-C); ^1H NMR($CDCl_3$, 300 MHz): 2.42 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.27 (s, 1H, CH), 6.59 (d, 1H, *J*=8.1 Hz), 6.73 (d, 1H, *J* = 8.4 Hz), 7.24 (d, 1H, *J* = 8.4 Hz), 7.40 (d, 1H, *J*=1.2 Hz), 7.43 (d, 1H, *J*=1.5 Hz).

3,3'-(4-Thiomethyl benzylidene)-bis (8-methyl-4-hydroxycoumarin) 4g . 8-Methyl-4-hydroxycoumarin (0.02 mole, 3.52g) was dissolved in 30 mL of ethanol and heated on a water-bath, just to get clear solution. 4-Thiomethylbenzaldehyde (4-methylmercaptobenzal-

Table II—Antiviral (HIV - 1 & HIV - 2) activity data

Compd Code	HIV Strains used	EC_{50} Prot.	EC_{90}	CC_{50}	SI	Max.
3b	IIIB	>49	>49	48.6	<1	0
3b	ROD	>12	>12	11.5	<1	2
3c	IIIB	>16	>16	16.2	<1	0
3d	IIIB	>10	>10	10.1	<1	9
3e	IIIB	>8	>8	8.4	<1	4
3f	IIIB	>125	>125	>125	><1	4
3g	IIIB	>12	>12	12	<1	8
3h	IIIB	>10	>10	10.1	<1	2
3i	IIIB	>12	>12	11.9	<1	3
3j	IIIB	>12	>12	12.5	<1	2
3k	IIIB	>45	>45	45.4	<1	6
4b	IIIB	>54	>54	53.7	<1	18
4c	IIIB	>9	>9	8.9	<1	1
4d	IIIB	>12	>12	12	<1	11
4e	IIIB	>12	>12	11.7	<1	7
4f	IIIB	>9	>9	9.1	<1	3
4g	IIIB	>13	>13	13	<1	3
4h	IIIB	>10	>10	9.8	<1	0
4i	IIIB	>10	>10	10.3	<1	3
4j	IIIB	>12	>12	12	<1	3

EC_{50}=Effective concentration at 50 µg/mL; EC_{90}=Effective concentration at 90 µg/mL; CC_{50}=Cytotoxic concentration at 50 µg/mL; IIIb = HIV – 1 Strain, ROD = HIV – 2 Strain ; SI = Selectivity Index; Max Prot. = Maximum Protection.

dehyde) (0.01 mole, 1.52 g) was added to the hot solution and refluxed for 18 hr. The solvent was distilled off and 3,3'-(4-thiomethyl benzylidene)- bis (8-methyl-4-hydroxycoumarin) separated out as crystals. It was recrystallized from ethanol as pale yellow crystals. m.p. 260-62°C (yield 62%); IR (KBr) : 3439 (OH), 1662 (>C=O, coumarin), 1226 (C-O-C), 846 cm^{-1}(C-S-C); ^1H NMR(300 MHz, $CDCl_3$): 2.45 (s, 3H, SCH_3), 6.05 (s, 1H, CH), 7.11 (d, 1H, *J*=8.7Hz), 7.18 (d, 1H, *J*=1.5Hz), 7.20 (d, 1H, *J*=1.8Hz), 7.26 (d, 1H, *J*=6.9Hz), 7.45 (d, 1H, *J*=7.2Hz). Anal. Calcd. for $C_{28}H_{22}O_6S$: C, 69.14; H, 4.53; S, 6.58. Found: C, 69.16; H, 4.50; S, 6.60%.

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