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SYNTHESIS OF PHENACYL DERIVATIVES OF FRANGULA-EMODIN AND THEIR HIV-1 RNase H ACTIVITY

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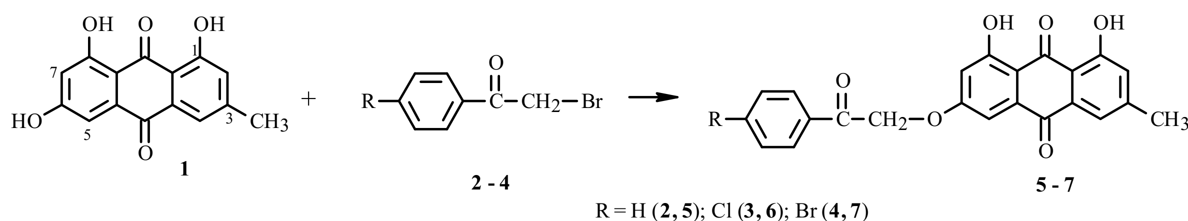
New functionally substituted phenacyl derivatives of frangula-emodin were synthesized and tested as inhibitors of HIV-1 RNase H activity.

Key words: frangula-emodin, phenacyl derivatives, HIV-1 RNase H activity.

Anthraquinones are widely distributed in nature and have been observed in higher plants, fungi, mosses, and lichens and are produced by bacteria [1]. 1,6,8-Trihydroxy-3-methylanthraquinone (frangula-emodin) (**1**) is one of the active ingredients of various plants. An investigation of its biological activity found antibacterial and anti-inflammatory activity [2, 3] and a capability to inhibit proteinkinase CK2 [4] and to interact with DNA [5–7]. It was also described as an inhibitor of cytomegalovirus [8] and hepatitis B virus [9]. Frangula-emodin and its analogs exhibit antifungal, anti-inflammatory, hormonal [10–14], and antitumor activity [15, 16].

Rumex tianschanicus A. Los [17] has been used as starting plant material for producing frangula-emodin, for which physicochemical and spectral data agreed with that published [18] and correlated well with the known literature [1, 19].

We used alkylation by bromoketones **2**–**4** to synthesize new derivatives of frangula-emodin. It has been shown [18] that carrying out the reactions in acetone in the presence of potash leads to substitution of the β-OH group to form monosubstituted compounds.



The purity of the resulting derivatives **5**–**7** was confirmed by TLC; identification, by elemental analysis and UV, IR, and PMR spectra. The compounds were crystalline and soluble in polar organic solvents and insoluble in water.

IR spectra of **5**–**7** contained stretching vibrations of carbonyls in the 9- and 10-positions of the anthraquinone system at 1672-1609 cm^{-1} and of the 6-substituent at 1696-1688. Stretching vibrations of C=C in aromatic rings were observed at 1586-1584 cm^{-1}.

Introduction of the phenacyl fragment on the 6-OH was confirmed by the lack of a resonance in PMR spectra for the β-OH proton. Resonances of α-OH protons were found at 11.97–13.32 ppm. Resonances at 6.70–8.00 ppm corresponded to aromatic protons of the phenacyl fragment and the α- and β-protons of the anthraquinone. Protons of (C-3, CH_3) and (–CH_2–) groups resonated at 2.40–2.45 and 5.40–5.86 ppm, respectively.

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TABLE 1. Effect of β-Phenacyl Derivatives of Frangula-Emodin on HIV-1 RNase H Activity

Compound	[a]IC_{50}, μM	[b]CC_{50}, μM
	HIV-1 RNase H	K 562
1	75	35±5
5	>100	>100
6	91	[c]ND
7	29	[c]ND

[a]Concentration of derivative required to reduce HIV-1 RNase H activity by 50%; [b]concentration of derivative required to reduce K562 multiplication by 50%; [c]no data.

Discovery of new inhibitors of HIV-1 RT is a crucial problem for creating anti-AIDS drugs. Strong and selective inhibitors of enzymes such as reverse transcriptase (RT) and protease (PR) are used in clinical practice to treat HIV-1 infection. RT of HIV-1 catalyzes transformation of viral RNA into double-stranded DNA. RT fulfills two distinct roles in carrying out this process. The first is DNA polymerase activity, which recognizes RNA and DNA templates; the second, destructive activity called RNase H, which hydrolyzes the RNA component of the hetero-doublet RNA:DNA intermediate. Synthesized phenacyl derivatives of frangula-emodin **5**–**7** were tested for their ability to inhibit HIV-1 RNase H activity in biochemical tests. Table 1 gives the test results.

The results show that starting derivative **1** inhibited weakly HIV-1 RNase H activity in biochemical tests. Introducing the phenacyl substituent on the β-OH made derivative **5** inactive. However, tests of derivatives **6** and **7**, which have a halogen in the 4′-position of the phenacyl fragment, showed that introducing a Cl atom reduced the minimal inhibiting activity whereas introducing a Br atom increased the inhibiting activity by 2.5–3 times.

These results indicated that new anti-RNase H inhibitors can be developed based on natural frangula-emodin. Modeling of their interaction in the derivative–enzyme complex by computer methods will enable the creation of more powerful new compounds with HIV-1 RNase H activity.

EXPERIMENTAL

The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using various solvent systems. UV spectra were recorded on a Lambda 35 UV/Vis spectrometer (Perkin–Elmer); IR spectra, in KBr disks on a Nicolet 5700 spectrometer; PMR and ^{13}C NMR spectra, on a Bruker-500 spectrometer at room tmeperature with TMS internal standard; mass spectra (EI, 70 eV), in a Finnigam MAT 8200. Melting points were measured on a Boetius apparatus. Products were separated over silica gel (Silica Gel 60, Merck, Germany) with elution by hexane:EtOAc (gradient from 100:0 to 40:60 v/v). Elemental analyses of the derivatives agreed with those calculated. Testing of the synthesized compounds as inhibitors of RNase H activity was carried out as before [20]; cytotoxicity, by the literature method [21].

Preparation of 5-7. A solution of frangula-emodin (**1**, 2.7 g, 0.01 mol) in acetone (100 mL) at room temperature was treated with the appropriate bromoketone (**2**–**4**, 0.01 mol) and K_2CO_3 (1.38 g, 0.01 mol). The reagents were mixed. The mixture was refluxed at about 45–50°C for 3–15 h. After the reaction was finished, part of the solvent was distilled. The mixture was treated with water and acidified with HCl. The resulting precipitate was filtered off and dried. The products were isolated using column chromatography over silica gel (L 40/100) with elution by hexane:EtOAc (gradient from 1:1 to 0:100 v/v).

6-*O*-Phenacyl-1,8-dihydroxy-3-methylanthraquinone (5). $C_{23}H_{16}O_6$, mp 201–203°C, R_f 0.95, C_6H_6:acetone (2:1), 0.59 CCl_4:Et_2O (5:2). UV spectrum (CH_3CN, λ_{max}, nm): 224, 247, 266, 280, 428. IR spectrum (KBr, ν, cm^{-1}): 1696 ($C=O_{sub}$), 1672, 1624 ($C=O_{anth}$), 1584 (C=C, Ar). Mass spectrum (EI, 70 eV, *m*/*z*): 388 $[M]^+$, 223 (32), 105 (100), 77 (75), 51 (30).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 2.40 (s, C$\mathbf{H}_3$-3), 7.01 (1H, d, J = 2.4, H-7), 7.15 (1H, s, H-2), 7.29 (1H, d, J = 2.7, H-5), 7.46 (1H, s, H-4), 13.11 (1H, s, α-OH), 13.32 (1H, s, α-OH); protons of 6-substituent: 5.84 (2H, s, –CH_2–), 7.57 (2H, m, >CH–, Ar), 7.70 (1H, m, >CH–, Ar), 8.00 (2H, m, >CH–, Ar).

6-*O*-(4′-Chlorophenacyl)-1,8-dihydroxy-3-methylanthraquinone (6). $C_{23}H_{15}ClO_6$, mp 223–225°C, R_f 0.92, C_6H_6:EtOAc (4:1), 0.81 hexane:EtOAc (2:1). UV spectrum (CH_3CN, λ_{max}, nm): 223, 255, 285, 305, 433. IR spectrum (KBr, ν, cm^{-1}): 1686 ($C=O_{sub}$), 1625, 1610 ($C=O_{anth}$), 1585 (C=C, Ar). Mass spectrum (EI, 70 eV, *m/z*): 422 $[M]^+$, 139 (100), 125 (4), 111 (25), 75 (15), 50 (7).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.45 (3H, s, C**H**$_3$-3), 6.70 (1H, d, J = 2.57, H-7), 7.10 (1H, s, H-2), 7.38 (1H, d, J = 2.57, H-5), 7.62 (1H, d, J = 2.0, H-4), 12.05 (1H, s, α-OH), 12.26 (1H, s, α-OH); protons of 6-substituent: 5.40 (2H, s, CH_2), 7.52 (2H, dd, >CH–, Ar), 7.94 (2H, dd, >CH–, Ar).

6-*O*-(4′-Bromophenacyl)-1,8-dihydroxy-3-methylanthraquinone (7). $C_{23}H_{15}BrO_6$, mp 228–230°C, R_f 0.94, C_6H_6:EtOAc (4:1), 0.83 hexane:EtOAc (2:1). UV spectrum (CH_3CN, λ_{max}, nm): 223, 256, 285, 305, 434. IR spectrum (KBr, ν, cm^{-1}): 1688 ($C=O_{sub}$), 1626, 1609 ($C=O_{anth}$), 1586 (C=C, Ar). Mass spectrum (EI, 70 eV, *m/z*): 467 $[M]^+$, 253 (5), 183 (100), 169 (10), 155 (20), 139 (22), 115 (7), 76 (15), 50 (10).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 2.44 (3H, s, C**H**$_3$-3), 6.99 (1H, s, H-7), 7.22 (1H, s, H-2), 7.27 (1H, s, H-5), 7.55 (1H, s, H-4), 11.97 (s, α-OH), 12.15 (s, α-OH); protons of 6-substituent: 5.86 (2H, s, $–CH_2–$), 7.80 (2H, dd, >CH–, Ar), 7.95 (2H, dd, >CH–, Ar).

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