Inhibition of reverse transcriptase and Taq DNA polymerase by compounds possessing the coumarin framework

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ABSTRACT

Coumarin derivatives were prepared using natural products isolated from plants belonging in the Pterocaulon genus (Asteraceae) and commercial drugs. Some molecules have displayed interesting activity against myeloid murine leukemia virus-reverse transcriptase (MMLV-RT) (compounds 20 and 28 produced inhibition with IC50 values of 38.62 and 50.98 µM, respectively) and Taq DNA polymerase (analogues 13 and 14 produced inhibition with IC50 values of 48.08 and 57.88 µM, respectively). Such inhibitors may have importance as antiretroviral chemotherapeutic agents and also in the development of anticancer drugs.

Keywords:
Coumarins
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Molecular target
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Human Immunodeficiency Virus (HIV) is the viral agent of acquired immunodeficiency syndrome (AIDS), and at present, there is no effective vaccine against HIV.1 Reverse Transcriptase (RT) is an essential enzyme for retroviral replication, such as HIV as well as for other RNA infectious viruses like Human T lymphocyte virus.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), a class of antiretroviral chemotherapeutic agents, act by binding to an allosteric pocket showing, generally, low toxicity.3 Another potential molecular target is DNA polymerases, which represent important cellular targets in the development of anticancer and antiviral agents.4

Natural coumarins have attraction due to bioactive properties such as tumor promotion inhibitory effects,5 anti-bacterial activity,6 antituberculosis,7 anti-influenza,8 anti-inflammatory,9 and as anti-coagulants.10 Coumarins and derivatives exhibit potent inhibitory effects on HIV-1 replication in H9 lymphocytes and compounds isolated from Calophyllum inophyllum showed inhibitory activity against human RT.11,12 Furthermore, natural isocoumarins isolated from cultures of fungi were able to inhibit human DNA polymerase 1.13 The object of the present work was to examine the ability of compounds possessing the coumarin framework to inhibit RT from myeloid murine leukemia virus (MMLV) and Taq DNA polymerase.

For the present study we selected two plant species from genus Pterocaulon (Asteraceae); P. virgatum (L.) D.C and P. alopecuroides (L.) D.C, known as coumarin’s producers.14,15 We describe the isolation and characterization of four coumarins: sabandinine 1; 5-(3,3-dimethylallyllyloxy)-6,7-methylenedioxycoumarin 2; 7-(3’-methyl-2,3’-dihydroxybutoxy)-6,8-dimethoxy coumarin 3 and 7-(3’-methyl-2,3’-dihydroxybutoxy)-6-methoxycoumarin 4 (Fig. 1).

We evaluate the potential antiviral therapeutic properties from natural and semi-synthetic analogues derivatives. The family of compounds was screened by retro-transcription polymerase chain reaction (RT-PCR) and classical polymerase chain reaction (PCR) inhibition.16

In order to increase the number of compounds for biological tests we performed chemical transformation over products 2 and 4. Thus, 7-(3’-methyl-2,3’-dihydroxybutoxy)-6-methoxycoumarin (4) was treated with H2SO4 in presence of acetone, leading to the formation of compound 5, with a typical acetonide moiety with a yield of 93% (Scheme 1). This group is also present in natural coumarins 1 and 2, resembles in part their structure.

Besides, acetylation of hydroxyl groups in compound 4 was carried out in a regio-selective manner. Thus, secondary hydroxyl group was preferentially acetylated (7) to tertiary hydroxyl (6) when the reaction was carried out in the absence of DMAP. Using DMAP as a catalyst the per-0-acetylated derivative (6) was obtained as the sole product, with 94% yield (Scheme 1).

In another group of reactions the γ,γ-dimethylallyl moiety present in compound 2 was subjected to epoxidation with m-CPBA to
and ammonium salt to achieve O values of 48.08 ± 9.26 and as alkyl-O.

The dihalogenated product initially obtained could suffer SN1 expected dibromoderivative. This result can be explained in case 14. Surprisingly, halohydrin chloride or acetic acid furnished a complex mixture of compounds. Simple addition of bromine performed with NBS in carbon tetrachloride or acetone, rt; (ii) 2.3 equiv Ac2O, Py, DMAP, rt; (iii) 1.0 equiv Ac2O, Py, 0 °C-rt.

In another set of reactions we used commercial 4-hydroxy-coumarin 15, 4-hydroxy-3-nitrocoumarin 16, coumaranone 17, 3-acetylcoumarin 18, 3-hydroxyxoumarin 19 and coumarin 20 to increase the number of derivatives for inhibition assays (Fig. 2). We were able to methylate the enol group of 15 using Me2SO4 as alkylation agent producing compound 21 (67% yield). In same substrate we can generate a benzoyl analogue (22) in a 57% yield, employing BzOCl. In order to incorporate a heteroatom on compound 15, it was subjected to reaction with methanesulfonyl chloride (23) (92% yield) and with p-chlorobenzoyl chloride (24) (78% yield) and both of them showed as white crystals (Scheme 3). However, compound 16 was only capable to reacting with this last protecting group, generating the derivative 25 (46% yield) (Scheme 4). This low reactivity of 16 may be explained by the high electro-drawing effect of the nitro group, which would decrease the nucleophilic character of the enol function. Finally, we proposed a prenylation reaction on 15 to achieve a structurally related derivative to the naturally 5-(3,3-dimethylallyl)-6,7-methylenedioxcoumarin 2. In this reaction, we obtained two products: the expected analogue 26 (59% yield) and a less polar dicarbonylic compound 27 (24% yield). Hybridization change and the incorporation of two isoprenoid chains, both on third position can be explained mainly by the substantial electron density present in this carbon and keto-enol equilibrium of 15. In the same way, we used allyl bromide in turbo cooler equipment, giving a mixture of compounds 28–30 with different degrees of substitution (Scheme 5). All synthesized compounds and natural coumarins isolated were well characterized by 1H and 13C NMR.

Different natural coumarins as well as synthetic analogs were found to display potent anti-HIV activity and can serve as potent NNRTIs in the development of anti-AIDS leads. It has been amply demonstrated that DNA polymerases and reverse transcriptases serve as molecular targets for antiviral chemotherapy. Assessment of the activity of Taq DNA polymerase was performed by PCR technique. In an initial screening we used a concentration equal to 500 nM; the results revealed that analogues 13 and 14 produced inhibition with IC50 values of 48.08 ± 9.26 and 57.88 ± 1.26 µM, respectively. Against expectations, the derivative bearing a Michael acceptor 12 was not active. However, the alcohol 13 obtained during the same reaction, was active. Compound 10
has a carbonyl group at side chain, and as 12 also shown inactive. On the other hand, the incorporation of bromide seems to be significant for protein recognition and inhibition,24–26 because compound 11 also have a tertiary alcohol and similar stereo-electronic factors as 14, but it was inactive. Compound 14 has a particular structural feature in their side chain. The bromine atom can act as a leaving group in a nucleophilic attack by nucleophilic residues of proteins. The presence of oxygen containing functional groups at both C-β can lead to neighboring group participation in the formation of the intermediate carbocation. This situation would result in a higher rate to the nucleophilic substitution (SN1) in relation to the intramolecular substitution reaction (SNi) giving rise to the epoxide. This center might be the determining factor in the inactivation of the enzyme. Moreover, in compound 12, where it would be possible to expect a conjugate Michael

Scheme 2. Reagents and conditions: (i) m-CPBA, K2CO3, Cl2CH2, 0 °C and dark conditions; (ii) H2, Pd/C, Cl2CH2, rt; (iii) 1.1 equiv KMnO4, THF/H2O (7:3), diethyl-ammonium fluoride, rt; (iv) 0.75 equiv SeO2, Cl2CH2, 2.0 equiv TBHP, 0–4 °C; (v) 2.2 equiv NBS, Cl2CH2, 0–4 °C.

Figure 2. Commercial coumarins used.

Scheme 3. Reagents and conditions: (i) Me2SO4, K2CO3, acetone, 56 °C; (ii) BzOCl, TEA, Cl2CH2, rt; (iii) 1.3 equiv MsCl, 1.2 equiv Py, 0.6 equiv DMAP, Cl2CH2, rt; (iv) 4-chlorobenzoyl chloride, TEA, Cl2CH2, rt.

Scheme 4. Reagents and conditions: (i) 4-Chlorobenzoyl chloride, TEA, Cl2CH2, rt.
addition, the oxygen atom of the ether function can be protected from nucleophilic attack by the proximity of their unshared electron pairs to the electrophilic carbon. This situation has previously been reported for α,β-un saturated cyclopentenone moieties in sesquiterpene lactones.27 Finally, the similar values of activity obtained with products 13 and 14 could be partially attributed to their related polarity.

Furthermore, we used all compounds obtained (except 13 and 14 because the RT-PCR experiment involve the Taq DNA polymerase activity) to evaluate reverse transcription process using a concentration of 500 μM for initial screening. Herein could be observed that compound 20 and 28 produced inhibition with IC50 values of 38.62 ± 3.25 and 50.98 ± 1.79 μM, respectively. These results indicate that in reverse transcription, simple molecules like coumarin and 28 are able to inhibit this event. Taking into account the structure, it is hard to think that coumarins act recognizing the enzymatic active site. Probably they can place allosterically, for example at some hydrophobic pocket.28 Furthermore, it is interesting to note that none of the naturally occurring compound was active against Taq DNA polymerase and RT-MMLV. However, these compounds are important synthons and skeletons for obtaining active compounds. Also, it is possible to observe that the compounds inhibit the DNA polymerase present methylenedioxy ring; different from that observed with RT inhibitor compounds which do not possess the mentioned moiety. These results convert to the methylenedioxy ring in necessary for activity against Taq DNA polymerases but it is not the only required condition because compounds as 1–2 and 10–12 are not active.

The aim of the present study was to evaluate the inhibition by coumarin, because of these compounds have showed important inhibitory activity against HIV-infected lymphocytes.29 There is a high relationship between RNA dependent enzymes as RT-MMLV and DNA dependent polymerases like Taq polymerase. These structures resemble a right hand domain and conserve amino acid sequence inside catalytic region.23 We also found that some coumarins can inhibit the Taq DNA polymerase activity and in this way could be cataloged as new polymerase inhibitors (see Table 1). Taking into account the results from this Letter, it is possible to say that the coumarin analogs merit special attention as potential antivirals.

In summary, we have isolated four natural coumarins from P. virgatum and P. alopecuroides and prepare twenty semi-synthetic analogues besides commercial drugs. Their inhibitory activity against Taq DNA polymerase and MMLV-RT was evaluated and two molecules (compounds 13 and 14) with similar structures showed inhibitory activity against Taq DNA polymerase. Also, coumarin (20) and compound 28 were able to inhibit the reverse transcriptase with interesting values of activity. Thus, novel leads from these coumarins can be further developed into potential chemotherapeutic agents in antiviral treatment.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.104.
H NMR (200 MHz, CDCl3) 1.82 (s, 3H), 4.11 (s, 2H), 4.95 (d, 2H, J = 6.8 Hz), 5.77 (s, 1H, J = 4.4 Hz) 6.02 (s, 2H), 6.18 (d, 1H, J = 9.6 Hz), 5.64 (s, 1H), 7.96 (d, 1H, J = 9.6 Hz); 13C NMR from HSQC (50.6 MHz, CDCl3) 26.00, 25.60, 61.57, 71.11, 75.46, 96.90, 117.99, 138.93.

5-(3-Methyl-2,3-dihydroxybutyroxy)-6-methylenoxocoumarin (14): 1H NMR (200 MHz, CDCl3) 1.44–1.25 (br m, 6H), 3.34 (br m, 1H, J = 3.2; 5.6 Hz) 4.50 (dd, 1H, J = 8.8; 2.8 Hz), 5.02 (dd, 1H, J = 3.2; 8.0 Hz) 6.05 (s, 2H), 6.22 (d, 1H, J = 9.8 Hz), 6.56 (s, 1H), 8.12 (d, 1H, J = 9.8 Hz); 13C NMR from HSQC (50.6 MHz, CDCl3) 30.00, 64.20, 74.10, 93.22, 101.90, 111.92, 139.80.

2-H-1-Benzopirany-2-one (20): 1H NMR (200 MHz, CDCl3) 6.45 (d, 1H, J = 9.4 Hz), 7.31 (br m, 1H), 7.35 (d, 1H, J = 8.1 Hz), 7.52 (d, 1H, J = 7.8 Hz), 7.56 (br m, 1H), 7.73 (d, 1H, J = 9.4 Hz); 13C NMR from HSQC (50.6 MHz, CDCl3) 115.99, 116.18, 123.72, 127.17, 131.12, 142.73.

4-Hydroxy-3-alloyoxocoumarin (28): 1H NMR (200 MHz, CDCl3) 3.50 (d, 2H, J = 6.8 Hz), 5.27 (br m, 2H), 6.03 (br m, 1H), 7.27 (br m, 2H), 7.31 (dd, 1H, J = 1.6; 7.2 Hz) 7.81 (d, 1H, J = 8.0 Hz); 13C NMR from HSQC (50.6 MHz, CDCl3) 29.00, 116.15, 117.20, 121.89, 123.05, 134.23, 135.76.