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SYNTHESIS OF 5,8-DIMETHOXY-3-HYDROXY-4-QUINOLONE, A REPORTED INHIBITOR OF HIV RT, AND EVIDENCE THE ORIGINAL PROPOSED STRUCTURE WAS INCORRECT

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Abstract: An unambiguous total synthesis of the title compound, a semi-synthetic derivative reported to be a non-nucleoside reverse transcriptase inhibitor, was conducted in four steps from 2,5-dimethoxyaniline. The synthetic material differed from that reported in the literature, both in its physical properties and ^{1}H NMR spectrum. Biological evaluation indicated that synthetic **2** was inactive against HIV-1 RT, suggesting that the previous structural assignment of the semi-synthetic derivative was incorrect.

It was reported that 3,5,8-trihydroxy-4-quinolone (**1**, Figure 1), isolated from the Red Sea sponge *Verongia* sp., was a potent inhibitor of the reverse transcriptase enzymes from both HIV-1 and HIV-2.[1] The compound inhibited HIV-1 RT in a non-competitive manner, with an inhibition constant (K_i) of 0.49 μM. Using a non-denaturing gel retardation assay, which measured the ability of RT inhibitors to disrupt the complex formed between purified HIV-1 RT and a double-stranded DNA oligomer, it was determined that **1** likely binds HIV-1 RT at a different site than those bound by azidothymidine triphosphate (AZT-TP) or TIBO, a non-nucleoside RT inhibitor.[2] Neither AZT-TP nor TIBO interfered with enzyme-DNA complex formation, while the trihydroxyquinolone completely destabilized the complex, suggesting that this compound operates via a different mode of action than other RT inhibitors.

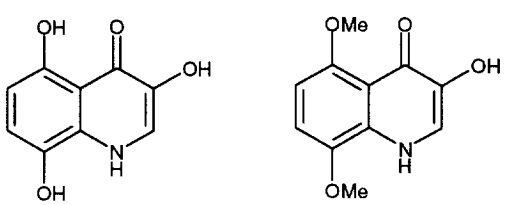

Figure 1

The trihydroxyquinolone **1**, which exists as a yellow pigment, was unstable to air and alkaline pH, rapidly forming an insoluble black material.[1] Another group, who had previously isolated **1** from the sponge

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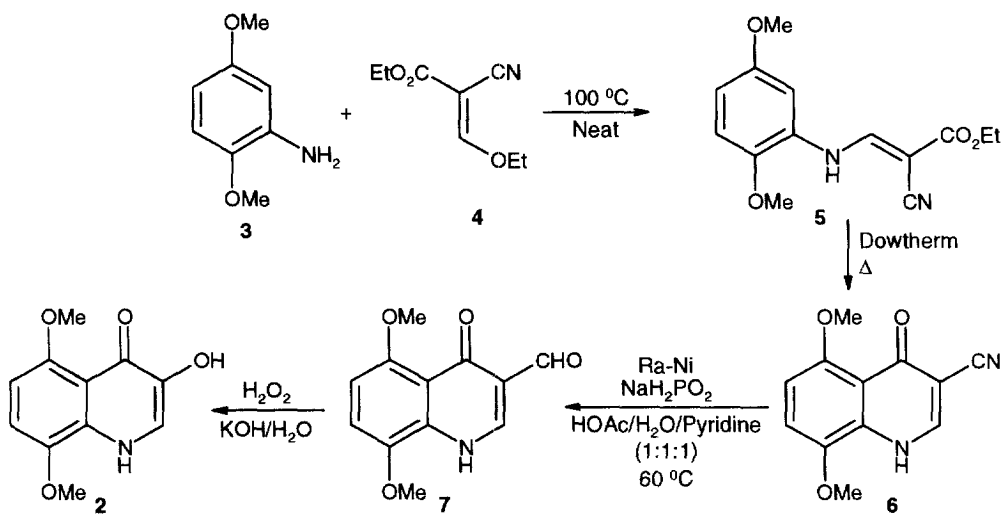
Verongia aerophoba, also reported this instability and concluded that the insoluble black material arose from oxidation of the 5,8-dihydroxybenzenoid ring to its corresponding quinone, followed by polymerization.[3]

Preparation of three semi-synthetic derivatives from the parent trihydroxyquinolone identified 5,8-dimethoxy-3-hydroxy-4-quinolone (**2**) as an analogue possessing nearly equal activity against HIV-1 and HIV-2 RT as the parent natural product, but with greatly improved chemical stability.[1] Their material **2** was prepared by treatment of **1** with iodomethane in acetone at room temperature. We chose to prepare **2** via total synthesis, which would provide a general synthetic route to examine the structure-activity relationships of this unique class of HIV RT inhibitor. No syntheses of either **1** or **2** have been previously reported.



Scheme

The desired compound **2** was prepared using the general route described by Goldsworthy and co-workers.[4] Starting from 2,5-dimethoxyaniline (**3**, Scheme), condensation with ethyl 2-cyano-3-ethoxyacrylate (**4**) afforded intermediate **5** in quantitative yield. Thermal cyclization of crude **5** in refluxing Dowtherm provided 5,8-dimethoxy-3-cyano-4-quinolone (**6**) in 80% yield (55% following recrystallization from methanol). Treatment of **6** with Raney nickel in the presence of sodium hypophosphite affected transformation of the nitrile to aldehyde **7**, in 21% yield. Finally, Dakin oxidation[5] of **7** afforded smooth conversion to the desired 5,8-dimethoxy-3-hydroxy-4-quinolone (**2**),[6] in 48% yield.

Upon completion of the total synthesis, it was noted that neither the physical properties nor the ^{1}H NMR spectrum of synthetic **2** matched those reported in the literature. Loya reported that **2** was isolated as a pale yellow oil,[1] whereas the material we obtained was a tan-colored, high-melting crystalline solid (mp 253-255 °C). A comparison of the ^{1}H NMR spectra, each recorded in DMSO-d_6, are presented in the Table.

The most striking difference between the two ^{1}H NMR spectra are the absorbances for the methoxyl groups. For our material, each methoxy group exhibited discreet singlets integrating for three protons each (δ 3.76 and 3.91). However, the literature reported that both methoxyl moieties absorbed at δ 3.40, as a singlet integrating for six protons.[1]

Table. Comparison of ^{1}H NMR spectra between synthetic **2** and literature values, recorded in DMSO-d_6

Proton	δ, Synthetic **2**	δ, Literature **2**
NH	10.92 (br s)	11.4 (br s)
H-2	7.46 (s)	7.70 (d, J = 4 Hz)
OH	Not visible	9.80 (br s)
OCH_3	3.76 (s, 3 H)	3.40 (s, 6 H)
	3.91 (s, 3 H)	
H-6	6.54 (d, J = 9.0 Hz)	6.25 (d, J = 8.0 Hz)
H-7	7.00 (d, J = 9.0 Hz)	6.95 (d, J = 8.0 Hz)

Because our synthesis began with 2,5-dimethoxyaniline, the sites which were methylated in the final quinolone **2** were unambiguously the 5- and 8-hydroxyls. However, the material described in the literature[1] was prepared from **1** by methylation with iodomethane at room temperature, which could potentially methylate at any of four different sites (1, 3, 5 and 8). Indeed, treatment of **1** with diazomethane produced 3,5,8-trimethoxy-4-quinolone, which was reportedly inactive against HIV RT.[1] No analytical data other than ^{1}H NMR and high resolution mass spectra were provided for **2**, so it is conceivable that methylation could have occurred at sites other than the 5- and 8-hydroxyls, as any of the possible dimethylated products would be expected to give similar ^{1}H NMR and mass spectra.

Our quinolone **2** was evaluated for inhibition of RNA-dependent DNA polymerase activity against wild type HIV-1 RT, using a poly(rC):oligo(dG)$_{12\text{-}18}$ template:primer system, as previously described.[7] The compound exhibited no inhibition of enzyme activity up to a concentration of 100 μM. This data, together with the discrepancies in physical properties and ^{1}H NMR spectra described above, indicate the structural assignment of the semi-synthetic material previously described[1] was likely incorrect.

Though there are four potential sites of compound **1** that could have been methylated (1, 3, 5 and 8), one can reasonably conclude that the 1-nitrogen was not methylated. Normally, basic conditions are necessary for *N*-alkylation of quinolones,[8] and the reported doublet for the H-2 hydrogen (J = 4 Hz) indicated coupling with the exchangeable proton on the 1-nitrogen. The lack of coupling between these two protons, however, is not a strong indicator that the nitrogen is not methylated, because a variety of 4-quinolones prepared by us often exhibited concentration-dependent coupling between the 1- and 2-protons (data not shown).

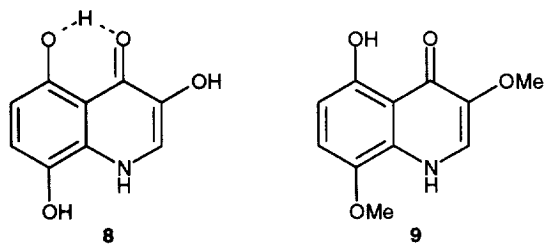


Figure 2

Alkylation of a phenolic hydroxyl having a *peri* relationship to a carbonyl is often sluggish, due to the stability of the six-membered hydrogen-bonded species **8** (Figure 2). Thus, a likely product formed via mild methylation of **1**, such as using iodomethane/acetone, would be 3,8-dimethoxy-5-hydroxy-4-quinolone (**9**). Further work is warranted to decisively determine the structure of the semi-synthetic analogue that possessed the interesting anti-HIV activity reported.[1]

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6. Mp 253-255 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (br s, 1 H), 7.46 (s, 1 H), 7.00 (d, 1 H, J = 9.0 Hz), 6.54 (d, 1 H, J = 9.0 Hz), 3.91 (s, 3 H), 3.76 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 170.7, 152.7, 142.4, 142.1, 131.2, 118.3, 114.3, 109.4, 101.8, 56.3, 56.1; APCI-MS *m/z* 222 (MH^+, 100), 207 (loss of -CH_3), 192 (loss of -CH_3); FTIR (KBr) 3200, 1554, 1520, 1426, 1262, 1064 cm^{-1}. Anal. calcd. for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.62; H, 4.99; N, 6.32.
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