Synthetic α-Hydroxytropolones as Inhibitors of HIV Reverse Transcriptase Ribonuclease H Activity

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Supporting Information

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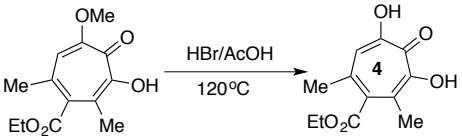
1. Compound Synthesis. Synthetic α-hydroxytropolones were synthesized from oxidopyrylium salt as described previously, and deemed >95% pure by ^{1}H NMR spectroscopy: (**1-3, 7, 8, 11, 15**);[1] (**20**)[2] (**9,10,12,14,16-22**);[3] (**5,13**)[4]

Compound **4** was synthesized by demethylation of the methoxytropolone precursor:

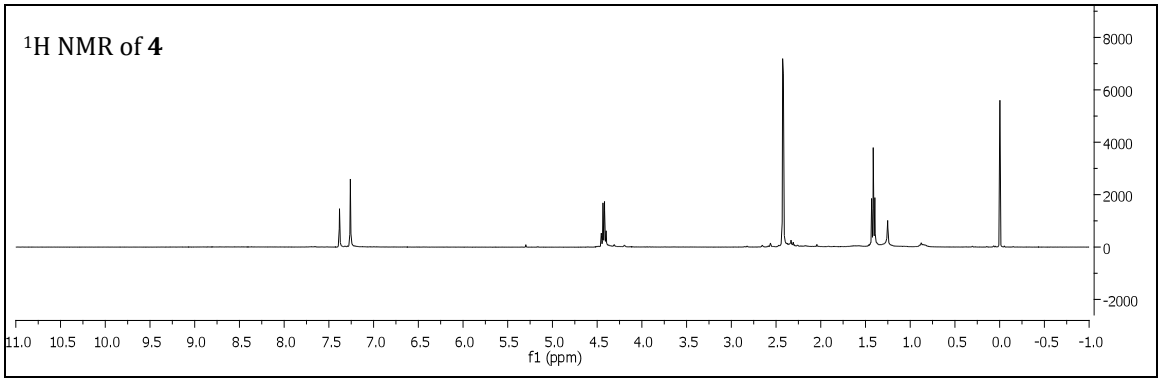


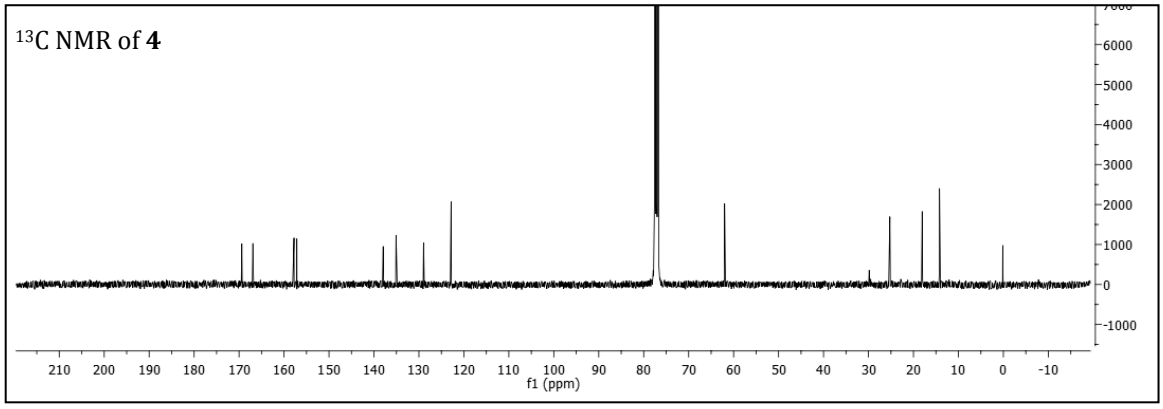
MT-4

In 512 μL of 33% HBr/AcOH solution was dissolved **MT-4**[1] (11.65 mg, 0.046 mmol). The reaction flask was fitted with a reflux condenser and allowed to stir at 120°C for 30 min, and then at room temperature for 10 min, before being quenched with phosphate buffer (pH 7). The organic layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **4** as a brown oil (5.8 mg, 53%). IR (thin film, KBr) 3243 (br), 2927 (w), 1727 (s), 1526 (m), 1448 (w), 1367 (m), 1282 (s), 1221 (s), 1061 (w), 1036 (m), 669 (w) cm-1. ^{1}H NMR (400 MHz, CDCl3) δ 7.38 (s, 1H), 4.43 (q, J = 7.1 Hz, 2H), 2.43 (s, 3H), 2.40 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 169.39 (s), 166.92 (s), 157.78 (s), 157.16

[1] Meck, C.; Mohd, N.; Murelli, R. P. *Org. Lett.* **2012**, *14*, 5988-91
[2] Williams, Y. D.; Meck, C.; Mohd, N.; Murelli, R. P. *J. Org. Chem.* **2013**, *78*, 11707-13.
[3] Hirsch, D. R.; Cox, G. C. ; D'Erasmo, M. P.; Shakya, T.; Meck, C.; Mohd, N.; Wright, G. D.; Murelli, R. P. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4943-4947.
[4] Masaoka, T.; Zhao, H.; Hirsch, D. R.; D'Erasmo, M. P.; Meck, C.; Varnado, B.; Gupta, A.; Mey-ers, M. J.; Baines, J. D.; Beutler, J. A.; Murelli, R. P.; Tang,L.; Le Grice, S. F. J. *Biochemistry*, **2016**, *55*, 809-19.

(s), 137.94 (s), 135.08 (s), 128.96 (s), 122.83 (s), 62.01 (s), 25.25 (s), 18.05 (s), 14.21 (s). HRMS (ESI+) m/z calc'd for C12H15O5+: 239.0914. Found: 239.0907.





2. RNase H Inhibitor Analysis. IC_{50} values were determined as previously reported[5] using an 18-nucleotide 3'-fluorescein-labeled RNA annealed to a complementary 18-nucleotide 5'-dabsyl-labeled DNA. Cleavage of the HIV-1 polypurine tract (PPT) primer was performed with a 29 nt Cy5-labeled RNA (5'-Cy5-UUU UAA AAG AAA AGGGGG G*AC UGG AAG GG-3', where *represents the PPT 3' terminus) hybridized to a 40 nt DNA (5'-ATT AGCCCT TCC AGT CCC CCC TTT TCT TTT AAA AAG TGG C-3'). The reaction was initiated by adding 1 µL of 100 mM MgCl2 to 9 µL of mixture containing 4 ng enzyme, 200 nM substrate, 20 µM α-hydroxytropolones in 50 mM Tris, pH 8.0, 80 mM KCl, 2 mM DTT, and 10% DMSO at 37°C and quenched with 10 µL of a gel-loading buffer after 10 min. Hydrolysis products were fractionated by denaturing polyacrylamide gel electrophoresis and visualized by fluorescent imaging (Typhoon Trio+, GE Healthcare).

[5] Budihas, S. R.; Gorshkova, I.; Gaidamakov, S.; Wamiru, A.; Bona, M. K.; Parniak, M. A.; Crouch, R. J.; McMahon, J. B.; Beutler, J. A.; Le Grice, S. F. J. *Nucleic Acids Res.* **2005**, *33*, 1249-56.

3. Differential Scanning Fluorimetry (ThermoFluor). Thermal stability assays were performed according to Nettleship et al.[6] To a LightCycler®480 96-well plate (Roche) was added 1 µL of 1 mM each compound (in DMSO), followed by 49 µL of DSF buffer containing 1.6 mM HIV RT RNaseH, 20 mM HEPES, pH 7.5, 10 mM MgCl2, 100 mM NaCl, and a 1:2000 dilution of Sypro® Orange dye (Invitrogen). The mixture was heated from 30 to 80°C in increments of 0.2 °C. Fluorescence intensity was measured using excitation/emission wavelengths of 483 nm and 568 nm, respectively. Changes in protein thermal stability (DTm) upon compound binding were analyzed by using LightCycler® 480 Software. All assays were performed in duplicate.

4. HIV-1 Cytopathicity Assay. This assay was conducted as previously reported.[7] Samples were dissolved in DMSO at 10 mM and diluted to a final high concentration of 50 µM in a 96-well assay plate, with 2-fold dilutions made to a low concentration of 0.78 µM. All samples were tested in duplicate. The HIV-1 virus strain RF was used to infect CEM-SS cells. Compound cytotoxicity was measured in the same assay plate using uninfected cells. Regression analysis was used to estimate the effective concentration (EC_{50}) as well as the cytotoxic concentration (CC_{50}).

5. Electronegativity Calculations. Mulliken electronegativities were computed as the negative of the average of the energy of the highest occupied molecular orbital (HOMO) and the energy of the lowest unoccupied molecular orbital LUMO.[8] The Gaussian program (Gaussian, Inc.) was employed to compute energies of the HOMO and of the LUMO using geometry optimization at the Hartree-Fock level with the 6-31g(d) basis set.

6. Alchemical binding free energy calculations. Binding free energies were computed with the Binding Energy Distribution Analysis Method (BEDAM).[9] The RNase H domain of HIV-1 reverse transcriptase was prepared from the PDB crystal structure 3K2P[10] using the Protein Preparation Wizard of the 2014 version of the Maestro program (Schrodinger, LLC) and the OPLS 2005 force field. Restraining potentials were applied to the metal cations to maintain them bound to to the active site residues. Ligand structures were prepared by using the LipPrep workflow within the Maestro program (Schrodinger, LLC). The two hydroxyl groups on the tropolone ring which contact the metal ions of the receptor, were modeled as deprotonated. Ionization penalties were computed at pH 7 with Epik (Schrodinger, LLC) and added to the BEDAM binding free energy estimates. The Glide

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[6] Nettleship, J. E.; Brown, J.; Groves, M. R.; Geerlof, A. *Methods Mol. Biol.,* **2008** , *426*, 299-318.
[7] Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. J. *Natl. Cancer Inst.* **1989**, *81*, 577–86.
[8] Chang-Guo Zhan, Jeffrey A. Nichols, and David A. Dixon. *J. Phys. Chem. A*, Vol. 107, No. 20, 2003 .
[9] Gallicchio, E.; Deng, N.; He, P.; Wickstrom, L.; Perryman, A. L.; Santiago, D. N.; Forli, S.; Olson A. J.; Levy, R. M. *J. Comp. Aided Mol. Design*, **2014**, *28*, 475-90.
[10] Himmel, D. M.; Maegley, K. A.; Pauly, T. A.; Bauman, J. D.; Das, K.; Dharia, C.; Clark, A. D. Jr.; Ryan, K.; Hickey, M. J.; Love, R. A.; Hughes, S. H.; Bergqvist, S.; Arnold, E. *Structure*, **2009**, *17*, 1625-35.

docking program (Schrodinger, LLC) was used to generate initial structures of the complexes. Glide receptor grid generation employed the structure of the receptor prepared as above. The crystallographic position of β-thujaplicinol in the 3K2P crystal structure was identified as the center of the docking search region. Positional constraints and metal coordination constraints were applied in Glide to chelate the the metal ions. The simulation temperature was set to 300K for all simulations. A total 18 intermediate alchemical states at λ =0.0, 0.002, 0.004, 0.008, 0.01, 0.02, 0.04, 0.07, 0.1, 0.17, 0.25, 0.35, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0, were employed. The binding site volume was defined as the conformation in which the center of mass of the ligand core was within 5.0 Å of the center of mass of the receptor site, where the center of mass of the ligand was calculated including the atoms of the tropolone ring only and the center of mass of the receptor site was calculated in terms of the Cα atoms of residues 443, 444, 478, 498, 539, 549, and 557. A flat-bottom torsional restraint potential in terms of the dihedral angle between the C1, C6 atoms of the ligand and the Cα atoms of residues 478 and 443 was applied to confine the ligand in one of the two orientations relative to the metal coordination plane. The two orientations were computed separately and the smaller binding free energy of the two was reported. Parallel BEDAM calculations were conducted using ASyncRE software[11] on the Brooklyn College WEB computing grid and the SuperMIC XSEDE supercomputing cluster.

7. Homology model of substrate- and inhibitor-bound HIV-1 RNase H. The docked model of RNase H bound to compound (1) was structurally aligned to the RNase H domain of the structure of full length HIV-1 reverse transcriptase bound to the DNA/RNA hybrid substrate (PDB id: 4B3O). Structural alignment was carried out by rigid-body minimization of the Root Mean Square Deviation (RMSD) of the positions of the Cα atoms of the corresponding residues using the Maestro program.

[11] Gallicchio, E.; Xia, J.; Flynn, W. F.; Zhang, B.; Samlalsingh, S.; Mentes, A.; Levy, R. M. *Computer Physics Communications*, **2015**, 196, 236–246.