Spectroscopic Studies of Delavirdine Mesylate (U-90,152T) a Bis(heteroaryl)piperazine (BHAP) HIV Reverse Transcriptase Inhibitor

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Complete spectral assignments, including mass spectral fragmentation pathways, ^{1}H and ^{13}C resonances, and major infrared absorbance bands for the BHAP (bis(heteroaryl)piperazine) HIV reverse transcriptase inhibitor delavirdine are reported.

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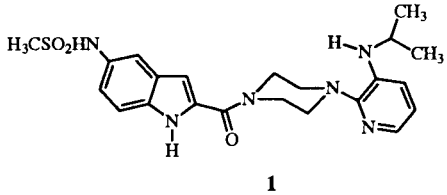
Retroviruses, such as the human immunodeficiency virus type 1 (HIV-1), are dependent on the enzyme reverse transcriptase (RT) to catalyze the conversion of viral RNA into proviral DNA. Reverse transcription is critical to the life cycle of HIV-1 and essential for replication of the virus [1]. Consequently, disruption of the reverse transcription through the inhibition of RT provides a therapeutic approach to the treatment of HIV infections. Several marketed drugs, including AZT [2], ddI [3], and related compounds, are nucleosides which compete with indigenous normal substrates and are in clinical use in the treatment of HIV-infected patients. Complications with the use of these therapeutic agents, and the emergence of resistant strains of the virus, have spurred the search for new therapeutic agents as well as new categories of compounds capable of inhibiting RT. Out of these efforts, the discovery and preliminary structure activity relationships (SAR) of the bis(heteroaryl)piperazine (BHAP) class of RT inhibitors was recently reported [4]. More recently, a more detailed SAR study of the BHAP RT-inhibitors was reported [5]. We now report the results of a detailed spectroscopic study of the BHAP RT-inhibitor, delavirdine mesylate (**1**, Rescriptor™) now in Phase III clinical trials.



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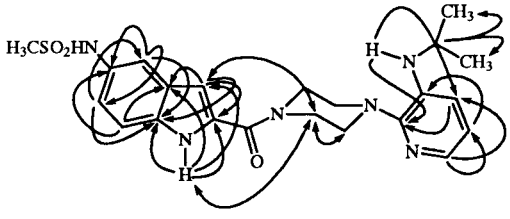
Unequivocal proton and carbon resonance assignments for **1** were obtained for the drug as both the free base and the bioavailable mesylate salt form used in the formulation of clinical trial dosage forms. The nmr spectra were acquired using samples of the free base or mesylate salt dissolved in d_6-DMSO. The nmr experiments were performed using either a Bruker AM- or AMX-400 operating at 400.13 MHz for ^{1}H observation or 100.25 MHz for ^{13}C observation. Proton and carbon reference spectra were assigned using a battery of two-dimensional nmr experiments which included COSY [6] or homonuclear TOCSY [7] to establish proton-proton connectivities, HMQC [8], or GHSQC [9] to establish direct or one-bond ($^{1}J_{CH}$) proton-carbon heteronuclear correlations, and GHMBC [10] to establish long-range proton-carbon coupling pathways. The latter experiment served to confirm assignments that could be tentatively made on the basis of chemical shift considerations, as well as providing an unequivocal means of making all other assignments, irrefutably locating heteroatoms, and establishing heteronuclear connectivities across intervening heteroatoms.

Complete proton and carbon resonance assignments for the free base and the mesylate salt are presented in Table 1. Briefly, the assignments made for the delavirdine free base sample will be described, as this is generally the spectroscopically preferred starting point for a study; the strategy used for the mesylate salt was identical. The isopropyl and sulfonamidomethyl resonances were assignable at 1.18 and 2.86 ppm, respectively, on the basis of chemical shift, multiplicity, and integration. The isopropyl methine and associated amino resonance were readily assigned to the resonances at 3.65 and 4.48 ppm, respectively, from homonuclear connectivity information. Within the pyridine system, the protonated three-spin system was assigned and correctly oriented using chemical shift arguments as well as a long-range heteronuclear coupling (HMBC) from the isopropyl amino NH group to the pyridine C4 resonance at 116.2 ppm. The pyridine H4/H5 resonances were overlapped at 6.92 ppm at 400 MHz, but by using HMBC correlations their respective carbon resonances, could still be unequivocally assigned. Using homo- and heteronuclear correlations from H4 and H5, the H6 resonance was assigned at 7.56 ppm, completing resonance assignments in the pyridine portion of the molecule.

Resonance multiplets associated with the piperazine ring were identifiable from chemical shift and integration as the

resonances at 3.01 and 3.93, but could not be unequivocally assigned from these data alone. Irradiation of the downfield piperazine methylene resonance gave nOe enhancements to the indole NH (11.64 ppm) and the indole H3 resonance (6.84 ppm) locating the methylene adjacent to the piperazine nitrogen attached to the carbonyl. (See double headed arrows on **2**). Within the indole system, H3, as just noted, was assignable from nOe difference data, as well as from the upfield shift of the directly bound carbon resonance (104.0 ppm) based on the HMQC spectrum. The H4 resonance at 7.48 ppm in the indolyl phenyl ring was readily assigned on the basis of its meta coupling to H6 which resonated at 7.10 ppm, which was, in turn, vicinally coupled to H7 resonating at 7.37 ppm. The sulfonamido NH resonance was assigned at 9.36 ppm; the sulfonamido methyl resonance was assigned at 2.86 ppm, as noted above, completing the assignment of all protons and protonated carbon resonances of delavirdine free base.

Assignment of the quaternary carbon resonances of the drug was straightforward from the long-range correlations observed in the HMBC spectrum. Rather than describing these assignments in detail, the observed correlation pathways are shown by **2**; correlations are denoted by unidirectional arrows from a given proton to the carbon to which it is long-range coupled; homonuclear nOes are denoted by double headed arrows.



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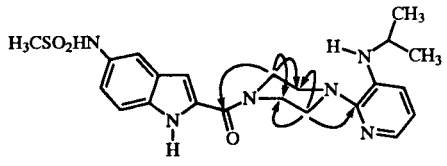
Responses corresponding to correlations in the piperazine ring were conspicuously absent from the conventional HMBC spectrum; their absence probably attributable to the broad nature of these proton resonances. This problem can, however, be circumvented, when necessary, by resorting to the very recently reported selective HMBC experiment described by Bax, Farley, and Walker [11]. Using this approach, it was possible to observe some of the long-range correlations arising from protons in the piperazine portion of the molecule. Selective pulses were applied to piperazine protons, giving the correlations denoted by **3**. To illustrate the advantages of using this approach, a segment of the contour plots of the conventional and selective HMBC spectra are shown in Figure 1. The selective pulse was applied to the piperazine protons nearest the point of attachment of the 2-pyridyl substituent.

Assignment of the proton and carbon spectra of the mesylate salt were conducted in an analogous fashion.



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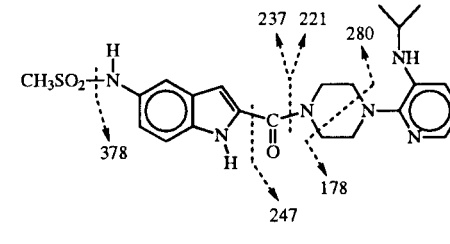
Complete proton and carbon resonance assignment for both forms of the molecule are presented in Table 1. It is worth noting similarities and differences. Proton and carbon chemical shifts within the indole and sulfonamidomethyl portions of the free base and mesylate salt were nearly identical. Considerable variance was noted, however, in the resonance positions for the piperazine and pyridine portions of the molecule because of the locus of mesylate salt formation. For this reason, all resonance assignments in both molecules were made rigorously and verified using connectivity information from the HMBC spectrum.

The FAB (fast atom bombardment) mass spectrum of **1** was studied using CID (collision induced dissociation), with argon as the collision gas at an indicated pressure of 1.5 torr, to increase the number of observable and potentially useful fragment ions in the spectrum. Data were acquired using a Finnigan TSQ-70 triple quadrupole mass spectrometer. By using this approach, in addition to the protonated parent ion, MH^+, observed at m/z = 457, a number of other fragment ions were observed arising from bond scission at key structural linkages. For example, the piperazinopyridine portion of the molecule was observed as an intact fragment ion at m/z = 221 along with the corresponding indole fragment with the carbonyl still attached to the 2-position of the indole nucleus at m/z = 237. The bond scission scheme accounting for the most intense fragment ions, with diagnostic potential for tracking metabolism and/or decomposition products of the drug, are shown by **4**.

High resolution mass spectrometry was also performed on a sample of delavirdine mesylate. The spectrum was recorded using electron ionization at a mass resolution of 10,000 (10% valley definition) and an ionization energy of 70 eV using a Kratos MS-50RF mass spectrometer. The measured accurate mass for delavirdine of 456.19454, which corresponds to an empirical formula of $C_{22}H_{28}N_6O_3S$, was within 0.18 ppm of theory. The exact



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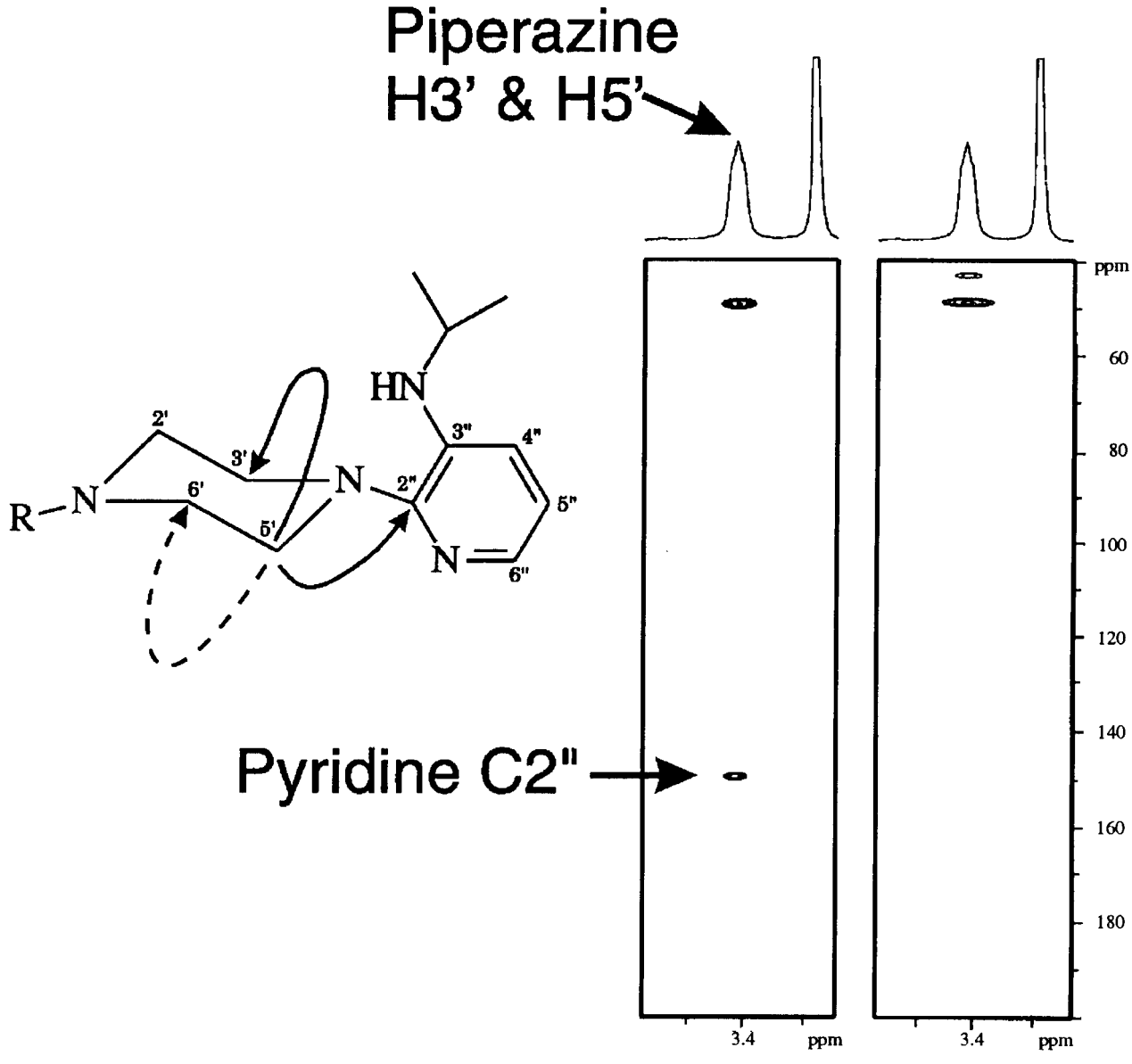

Figure 1. Conventional HMBC spectral segment [10] (right panel) selective HMBC spectral segment [11] left panel of delavirdine free base. The long-range correlation from the piperazine methylene protons to the quaternary C2 carbon in the pyridine ring is not observed in the conventional spectrum but is readily visible in the selective HMBC spectrum shown in the left panel. Dotted arrow selective HMBC; solid arrow conventional HMBC.

mass was measured by peak matching using the $C_{11}F_{17}$ ion of a perfluorokerosene reference at 454.97285.

The infrared spectrum of the molecule was recorded to provide a basis for future structural comparisons. The spectra were recorded with potassium bromide pellets using a Nicolet 60-SX FTIR. Key absorbances in the infrared spectrum of delavirdine mesylate were observed for: NH stretching (3370 and 3287 cm^{-1}); C=O, C=C, and C=N stretching (1625 and 1612 cm^{-1}); NH deformation bands (1558 and 1529 cm^{-1}); SO_2N stretching (1326 cm^{-1}); SO_3/C-N stretching (1235 and 1201 cm^{-1}); and SO_3/SO_2N stretching (1179, 1146, and 1043 cm^{-1}). In addition, bands were observed in the "fingerprint region" of the infrared spectrum for C-C and C-H bending (918, 876, 835, 788, 760, and 749 cm^{-1}). When interpreted in conjunction with the nmr and mass spectral data, the infrared data provided a useful and consistent supplement to the other conclusions drawn about the structure. A complete summary of the prominent infrared absorbance bands for delavirdine as both the free base and the mesylate salt, is presented in Table 2.

Table 1

Proton and Carbon NMR Chemical Shift Assignments of Delavirdine Free Base and Delaviridine Mesylate Recorded in d_6-DMSO

	Delavirdine free base		Delavirdine mesylate	
Functionality	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
i-Pr-CH_3	1.18	22.3	1.21	21.7
i-Pr-CH	3.65	42.9	3.70	43.4
i-Pr-NH	4.48	---	5.11 (br)	---
pyridine C2	---	149.6	---	146.9
pyridine C3	---	130.5	---	137.1
pyridine C4	6.92	116.2	7.63 [a]	127.0 [b]
pyridine C5	6.92	120.1	7.25	120.5
pyridine C6	7.56	134.3	7.34 [a]	120.9 [b]
3'/5'-piperazine CH_2	3.01	48.9	3.26	48.0
2'/6'-piperazine CH_2	3.93	42.3/40.5	4.00	44.0 [c]
indole NH	11.64	---	11.65	---
indole C2	---	130.9	---	130.6 or 130.3 [c]
indole C3	6.84	104.0	6.85	104.3
indole C3a	---	127.0	---	127.0
indole C4	7.48	114.4	7.49	114.4
indole C5	---	130.5	---	130.3 [c] or 130.6
indole C6	7.10	119.7	7.11	119.7
indole C7	7.37	112.7	7.40	112.6
indole C7a	---	133.7	---	133.5
C=O	---	161.9	---	161.8
5-N*H*SO_2	9 36	---	9.34	---
SO_2-CH_3	2.86	38.3	2.87	38.3
mesylate-CH_3	---	---	2.34	39.7

[a], [b] Assignments that may be permuted pairwise. [c] Observed at 72°C, not at 27°C ambient temperature.

Table 2

Correlation Table of Characteristic Infrared Bands and Corresponding Functional Groups of Delavirdine Free base and Mesylate Salt

	Delavirdine free base	Delavirdine mesylate
Functionality	Frequency (cm^{-1})	Frequency (cm^{-1})
O-H/N-H stretching (3600-3200)	3403 and 3126	3370 and 3287
C-H stretching	3100-2800	3100-2800
C=O, C=C, and C=N stretching	1597 (amide C=O) 1577, 1490	1625, 1612
N-H deformation	1525	1558, 1529
C-H deformation	1 480-1340	1480-1340
SO_2N stretching	1322, 1155	1326
SO_3/C-N stretching	-----	1235, 1201
SO_3/SO_2N stretching (1200-1000)	-----	1179, 1146, 1043
C-N stretching	1228, 1034	----
S-C-N stretching	979, 966, 957	975
fingerprinting C-C and C-H bending (1000-600)	929, 881, 792, 774, 759	918, 876, 835, 788, 760, 749

In conclusion, the total assignment of the nmr, mass, and infrared spectra of delavirdine free base and its mesylate salt provide a useful data base from which it is a reasonably facile process to identify the structures of metabolites, degradants, or possible synthetic process impurities. The latter could conceivably arise as new lots of raw materials are put into large scale synthetic production of the drug or as the commercial viability of new synthetic routes are examined. Metabolites could be studied from either *in vitro* enzymatic systems or *in vivo* systems ranging from various microbial species to patient volunteers. In contrast, it is probable under normal circumstances that it will be possible to study the structures of degradants only from samples subjected to highly stressed conditions during stability assurance testing that far exceed conditions that the marketed drug would ever be subjected to or expected to tolerate.

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† Analytical Research & Specifications Development.

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