Analysis of IR, ^{1}H NMR, and ^{13}C NMR Spectra of Binimetinib (Mektovi) Determined Using SPARTAN

CH-232: Organic Chemistry II

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Abstract

Binimetinib, an enzyme inhibitor created by Array Biopharma, is an anti-cancer molecule that inhibits key enzymes that cause melanoma and colorectal cancer. Binimetinib was analyzed using SPARTAN '18, a quantum chemistry software created by Wavefunction. The equilibrium geometry, Infrared Radiation (IR), Hydrogen Nuclear Magnetic Resonance (^{1}H NMR), and Carbon Nuclear Magnetic Resonance (^{13}C NMR) spectra are calculated using the Hartree-Fock model (HF) with a 6-31G* basis set. The IR spectrum determined by the HF calculation is analyzed by attributing various functional groups on binimetinib to peaks on the IR spectrum. The determined spectrum results in missing peaks and peaks with high wavenumbers between 3800 cm^{-1} and 3950 cm^{-1} that are not expected. The cause of this issue is likely due to the HF calculation determining the peaks at higher wavenumbers. The IR spectrum of binimetinib using the HF calculation method is not highly accurate for binimetinib. The ^{1}H NMR of binimetinib determined by the HF calculation results in peaks that have the expected multiplicity, chemical shifts, and integration as theoretically expected. The signal from a proton bonded to an oxygen atom is not determined by the HF calculation. The determined ^{1}H NMR spectrum results in accurate chemical shifts when compared to the chemical shifts of protons in similar compounds. The ^{13}C NMR spectrum determined by the HF calculation produces 17 signals for the 17 carbon atoms in unique chemical environments. The chemical shifts of these signals are similar to experimentally determined chemical shifts of carbon atoms of similar compounds and are within the theoretical chemical shift ranges. The ^{1}H NMR and ^{13}C NMR determined by the HF calculation is accurately determined for binimetinib.

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Introduction

Binimetinib is an enzyme inhibitor created by Array Biopharma that functions as an anti-cancer molecule that inhibits key enzymes that cause melanoma and colorectal cancer. The equilibrium geometry, Infrared Radiation (IR) spectrum, Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) spectrum, and Carbon Nuclear Magnetic Resonance (^{13}C NMR) spectrum can be determined using SPARTAN'18, a quantum chemistry software created by Wavefunction. The structure of binimetinib is shown in Figure 1, below.

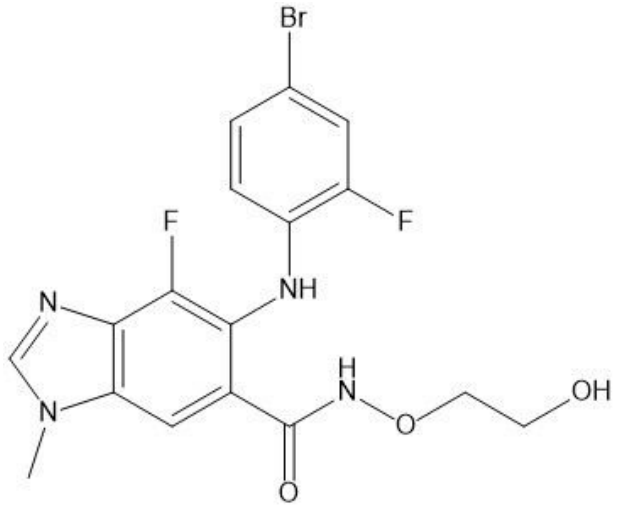

Figure 1. The bond line structure of Binimetinib [1].

Bonds in molecules absorb and release quantized energy, so a specific bond in a molecule only interacts with a small range of frequencies in the electromagnetic spectrum. When a bond absorbs the exact amount of energy required to promote vibrational excitation, the energy is temporarily stored and released. The released energy is also quantized and can be detected. IR spectroscopy takes advantage of the quantized storage and release of energy to determine the types of bonds that are present in a sample. The wavenumber, which is proportional to frequency, is used in IR spectra diagrams; so a higher wavenumber correlates to higher energy. The wavenumber range in which a type of bond will interact with the quantized energy is determined by the relative mass differences between the bonded atoms, bond length, intermolecular interactions, and the type of bond. Smaller masses, shorter bond lengths, and

bonds with more s character tend to absorb more energy. Intermolecular interactions such as hydrogen bonding causes the absorption spectrum to have broad peaks. A strong dipole moment in a bond results in higher intensity peaks [2]. The intensity, breadth, and wavenumber range of peaks in IR spectra is used to identify the types of functional groups present in compounds under inspection.

The protons and neutrons that make up the nuclei of atoms have a characteristic called nuclear spin. If there is an even number of protons and neutrons, the mass number is even, and the net spin in the nucleus is 0. However, when an atom has an odd mass number, there is a net spin, which results in the generation of a magnetic field called a magnetic moment. In the presence of an external magnetic field, the magnetic moment of each odd mass-numbered atom either aligns with the field (α state) or against the field (β state). The atoms in the β state have slightly more energy. Atoms can switch between α and β states by absorbing and releasing quantized energy that an instrument can produce and detect [2]. Nuclear Magnetic Resonance (NMR) spectroscopy takes advantage of the energy released by nuclei that are changing states to determine the chemical structure of compounds.

Since hydrogen atoms have an odd mass number of 1, ^{1}H NMR is frequently used to obtain information about the chemical structure of compounds. The three main attributes of ^{1}H NMR spectra are the chemical shifts, integration, and multiplicity. Electrons create a shielding effect that reduces the effect of an external magnetic field. Chemical shifts are a measure of how deshielded the nuclei of a protons are. A large chemical shift means that the nucleus of a proton is unprotected by its electrons because of electron delocalization or a neighboring electronegative atom. Integration is a measure of how many signals of the same type are present. Integration information allows chemists to identify the ratio of protons that have a specific type of signal. Multiplicity occurs when a proton interacts with its neighboring protons that are in either α or β state to create a combination of states with varying chemical shifts [2]. Multiplicity is useful in identifying the number of proton neighbors that are adjacent to the split proton.

Similar to ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy utilizes chemical shifts to determine the structure of chemical compounds. ^{13}C NMR can only detect ^{13}C isotopes because the most common isotope, ^{12}C, has an even mass number. The chemical shift of ^{13}C isotopes also

increases with an increase in deshielding due to electron delocalization or an electronegative neighbor [2].

The wave function calculations to produce IR, ^{1}H NMR, and ^{13}C NMR can be done using SPARTAN'18, a computer program that solves the Schrödinger equation numerically using various methods. The Hartree Fock (HF) calculation method assumes that each electron in an atom or molecule is impacted by all other electrons when performing the calculations [3]. The equilibrium geometry of binimetinib can be calculated using the HF calculation. In addition, the HF calculation can be used to determine the IR, ^{1}H NMR, and ^{13}C NMR spectra of compounds.

Procedure

SPARTAN ‘18 was used to determine the IR spectrum, ^{1}H NMR spectrum, and ^{13}C NMR spectrum for Binimetinib. The molecular structure for Binimetinib was recreated using SPARTAN’s sketch feature. Drawing a 2D molecule with the sketch feature automatically generated a ball and stick model. The sketch of Binimetinib was created by following the bond line structure of Binimetinib shown in Fig. 1.

The bond line structure was sketched starting with the benzene ring at the top in Fig. 1. The alkyl halides were then sketched. The nitrogen atom that connects the benzene ring and fused ring structure was sketched next. The fused ring structure was sketched, followed by the fluoro group and methyl constituent. The carbon chain on the bottom right of Fig. 1 was sketched last from the fused ring structure to the alcohol at the end of the chain. After sketching the bond line structure, the structure’s energy was minimized. The sketch was then exited to the main screen that showed the ball and stick model. The energy of the structure was minimized again. Then, the equilibrium geometry was calculated for Binimetinib in the ground state and gas phase with a 6-31G* basis set for the HF calculation. Binimetinib was set to a neutral charge with 0 unpaired electrons. The 6-31G* model with the Empirical (^{3} JHH) coupling constant was used to determine the IR, ^{1}H NMR. and ^{13}C NMR spectra. The IR spectrum determined by the HF calculation was analyzed to determine whether the peaks of the calculated IR spectrum were accurate to the theoretical IR peak range for various functional groups. The chemical shifts of the ^{1}H NMR and ^{13}C NMR spectra were also compared to theoretical chemical shift ranges to evaluate the accuracy of the calculated spectra for binimetinib.

Results and Discussion

Equilibrium Geometry

The equilibrium geometry of binimetinib determined by the HF calculation method is shown in Figure 2, below.

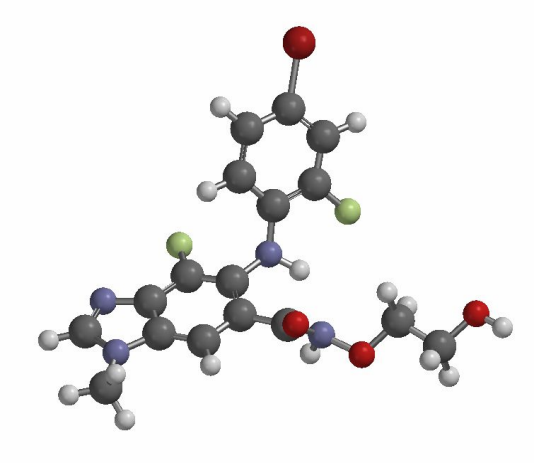

Figure 2. The equilibrium geometry determined by the HF calculation method.

IR Analysis

The functional groups that should theoretically appear in the diagnostic region of the IR spectrum are boxed and labeled with a letter in Figure 3, below.

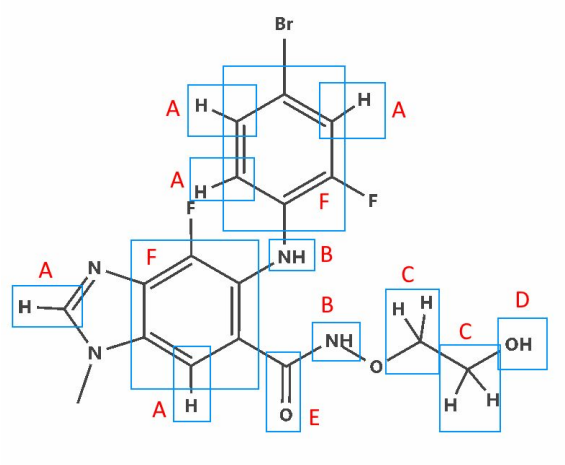

Figure 3. The functional groups of binimetinib that appear in the diagnostic region of the IR spectra boxed in blue and labeled in red [1].

Table 1, below, contains the expected wavenumber ranges in the diagnostic region for functional groups are present in binimetinib. The expected wavenumber range is based on Klein's *Organic Chemistry*.

Functional Group	Label	Expected wavenumber Range (cm^{-1})
H-C where the C is sp^2 hybridized	A	3000-3100
Primary Amine	B	3350-3500
H-C where the C is sp^3 hybridized	C	2850-3000
Hydroxyl	D	3200-3600
Carbonyl	E	1650-1700
Aromatic Structure	F	1650-2000

Table 1. The functional group with the expected wavenumber range for its corresponding peak based on Klein's Organic Chemistry.

Figure 4, below, shows the IR spectrum for binimetinib determined by the HF calculation.

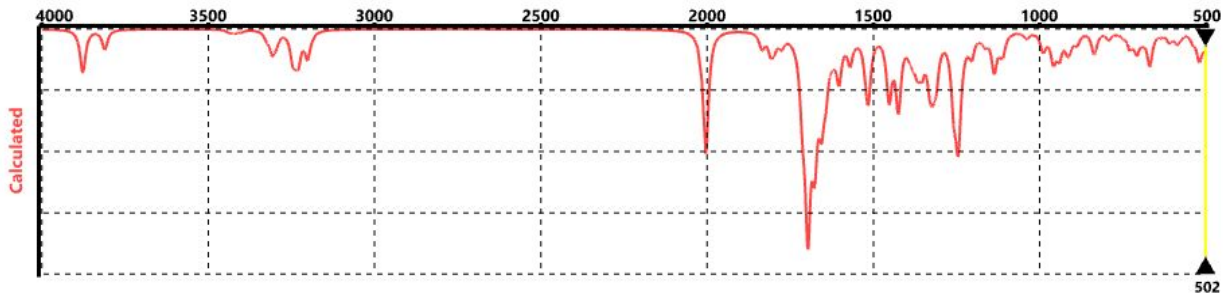

Figure 4. The IR spectrum of binimetinib determined by the HF calculation.

There is no peak between 3000 cm^{-1} and 3100 cm^{-1} even though there are 5 sp^2 hybridized C-H bonds (A). There is also no peak between 2850 cm^{-1} and 3000 cm^{-1} for sp^3 hybridized C-H bonds (C) even though there are 2 such bonds in binimetinib.

The amine peak (B) is expected to have 1 peak because there is only 1 hydrogen atom bonded to the nitrogen atom [2]. There are two peaks between 3350 cm^{-1} and 3500 cm^{-1}. One of the two peaks may be the hydroxyl group (D) with a wavenumber between 3200 cm^{-1} and 3600

cm^{-1}. A hydroxyl group usually has a large wavenumber range due to internal hydrogen bonding; but because binimetinib is sterically bulky, the hydroxyl group may not have as broad of a signal [2]. The peak closer to 3500 cm^{-1} is likely the amine peak because it is a single peak, and the peak closer to 3000 cm^{-1} is likely the hydroxyl peak.

A carbonyl peak is expected to have high intensity and should appear between 1680 cm^{-1} and 1750 cm^{-1} [2]. According to Fig. 4, there is a large peak at around 1700 cm^{-1}, which is likely the carbonyl signal. An aromatic group is expected to have a signal between 1650 cm^{-1} and 2000 cm^{-1} (see Table 1). As seen in Fig. 4, there is an intense signal at 2000 cm^{-1} that could account for the aromatic bonds.

Two signals are present around 3850 cm^{-1} to 3950cm^{-1}. Since the wavenumber is high, this is likely a hydrogen atom bonded to some electronegative atom; however, binimetinib does not have such electronegative atoms bonded to hydrogen, except for the primary amine and hydroxyl groups already mentioned. There is no functional group in binimetinib that can explain the peak at around 3850 cm^{-1}.

An explanation for the signals close to 4000 cm^{-1} is that the HF calculation produces an IR spectrum that has peaks that are overall shifted to higher wavenumbers than the theoretically expected range. The HF calculation may have calculated the peaks between 3850 cm^{-1} to 3950 cm^{-1} to be about 250 cm^{-1} higher than what is expected. One of the two high wavenumber peaks would belong to the primary amine and the other to the hydroxyl group. A shift lowering the determined wavenumbers could also explain the missing sp^2 and sp^3 hybridized C-H bonds. Lowering the calculated wavenumbers of 3350 cm^{-1} and 3500 cm^{-1} down by 400 cm^{-1} results in a range of 3100 cm^{-1} and 2950 cm^{-1}, which is the expected wavenumber range of sp^2 hybridized and sp^3 hybridized C-H bonds, respectively (see Table 1). The carbonyl signal and aromatic bond signals are on the higher end of the theoretical wavenumber range, so these may have also been calculated to have higher wavenumbers than expected.

After examining the IR spectrum produced by the HF calculation (see Fig. 4) and comparing the wavenumber range of the peaks of various functional groups to the expected wavenumber range of these functional groups, it is concluded that the HF calculation is not highly accurate at producing an IR spectrum. Although the theoretical wavenumber range of the

aromatic structure, hydroxyl group, carbonyl group, and primary amine group is within the theoretical wavenumber range of the IR spectrum produced by the HF calculation, the sp^2 and sp^3 C-H bonds of binimetinib did not have a signal. In addition, there are two unexplained signals around 3850 cm^{-1} that do not belong to any functional group in binimetinib. Therefore, the IR spectrum produced by the HF calculation is not highly accurate. It is possible that the HF calculation method determined the wavenumbers of these functional groups to be higher than what is expected.

^{1}H NMR Analysis

Figure 5, below shows the naming scheme of each carbon atom (C#), nitrogen atom (N#), and oxygen atom (O#).

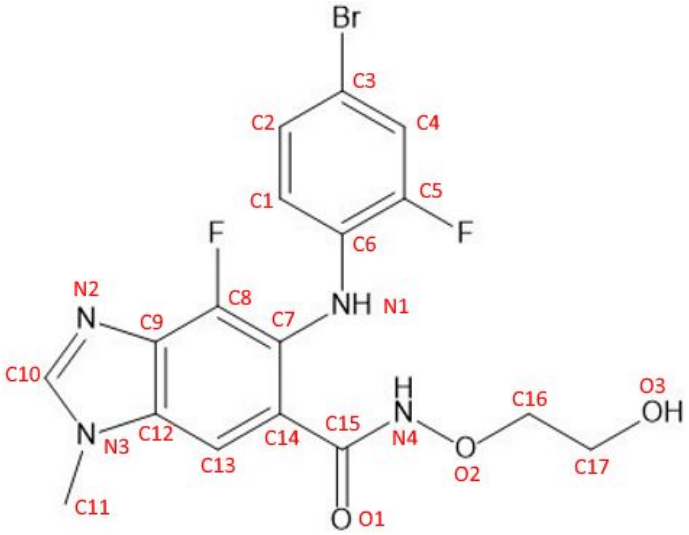

Figure 5. The structure of binimetinib with all carbon, nitrogen, and oxygen atoms labeled [1].

Figure 6, below, shows the structure of binimetinib with all of its hydrogens drawn using the SPARTAN molecule sketching tool. Each red rectangular box contains hydrogens that are chemically equivalent with respect to the other hydrogens in the red box.

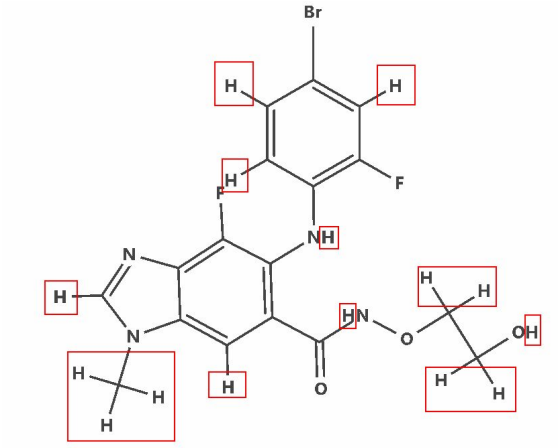

Figure 6. The structure of binimetinib with each unique hydrogen group boxed in red.

Based on the structure of binimetinib, there are 11 groups of hydrogen atoms that are chemically unique. Benimetinib lacks both rotational and reflectional symmetry, so there are no homotopic protons nor enantiotopic protons. Benimetinib is achiral, so there are no diastereotopic protons.

The protons attached to C1, C2, C4, C10, and C13 should have a large chemical shift likely around 6.5 ppm and 8 ppm because these carbon atoms are aromatic [2]. The protons on C2 and C4 should have a larger chemical shift than the protons on C1 because the protons on C2 and C4 neighbor an electronegative bromine atom, which causes an induction effect that increases the chemical shift. The chemical shift of the proton on N4 should be much greater than that of proton on N1 because the proton on N4 neighbors a carbonyl group and an oxygen atom. The protons on C16 and C17 should have a chemical shift of about 3.7 ppm because these protons both neighbor an oxygen atom [2]. C16 should have a slightly higher chemical shift than C17 because the proton on C16 is beta to an electronegative nitrogen atom. The proton attached to O3 should have a chemical shift between 2 ppm to 5 ppm [2].

Assuming there is no geminal hydrogen splitting, the protons attached to C1 and C2 should split each other into doublets since both the C1 proton and C2 proton neighbor only 1 proton. The proton attached to C4, C10, C11, C14, N1, N4, and O3 should be singlets because these protons have no neighboring protons. The protons on C16 and C17 should split each other into triplets because the protons in C16 and C17 neighbor 2 other protons on one side [2].

The integration of the signal from the protons on C11 should be the largest because the 3 protons bonded to C11 are chemically equivalent. The integration of the signal from the protons on C16 and C17 should be 2/3 of the integration of the protons on C11 because there exist two chemically equivalent protons attached to C16 and C17. All of the other signals should have an integration of a third of the integration of the protons on C11 because all of the other protons are in unique chemical environments.

Figure 7, below, shows the determined ^{1}H NMR spectra using the HF calculation method.

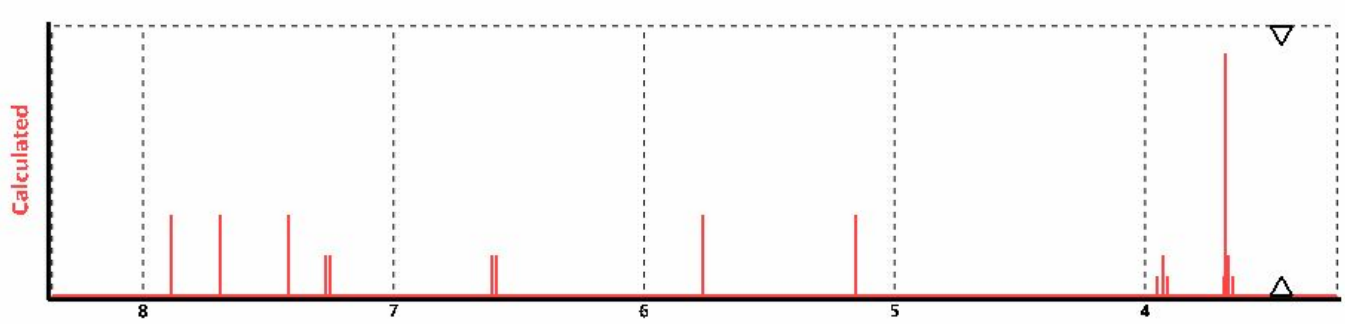

Figure 7. The ^{1}H NMR spectra determined using the HF calculation method.

There are 10 distinct signals determined by the HF calculation method. This is 1 less than the expected 11 signals because the HF calculation did not calculate the peak for the proton on O3. The tallest peak overlaps with a triplet at around 3.6 ppm. There are 6 singlets, 2 doublets, and 2 triplets. The two doublets correspond to the protons on C1 and C2. The two triplets correspond to the protons on C16 and C17. The rest of the protons are all singlets as expected.

The height of the C11 proton peak is approximately three times the height of the singlet peaks. The height of one part of a doublet peak is approximately half that of the singlet peaks, so the overall integration of a doublet peak is about the same as a singlet peak. The sum of the heights of a triplet peak is approximately the height of a singlet peak, but the total integration of the triplet peak is greater because the triplet peaks are spread over a greater chemical shift range. As expected, the integration of the triplet peaks are greater than that of a singlet peak.

The calculated chemical shift using the HF calculation method, the literature values of chemical shifts from similar compounds, and the theoretical chemical shift range is shown in Table 2. The literature chemical shifts are experimentally determined chemical shifts for compounds with similar chemical environments to different parts of binimetinib. The experimental ^{1}H NMR data is acquired from the National Institute of Advanced Industrial Science and Technology[4-6]. The theoretical chemical shift range is based on Klein's *Organic Chemistry* [2] and Starkey's ^{1}H NMR Chemical Shifts [7].

H Attached	Calculated Chemical Shift (ppm)	Literature Chemical Shift (ppm)	Theoretical Chemical Shift Range (ppm)
C1	6.59	6.628*	6.5 - 8
C2	7.25	7.031*	6.5 - 8
C4	7.42	7.123*	6.5 - 8
C10	7.89	7.828⊙	6.5 - 8
C11	3.68	3.805⊙	1 - 4
C13	7.69	7.25⊙	6.5 - 8
C16	3.93	3.716✦	~3.7
C17	3.67	3.716✦	~3.7
N1	5.15	-	0.5 - 5
N4	5.77	-	3.5 - 8.5
O3	-	3.17✦	2 - 5

Table 2: The ^{1}H NMR chemical shift determined by the HF calculation, the chemical shift, and the theoretical chemical shift for Binimetinib.

* based on ^{1}H NMR spectrum of 4-bromo-2-fluoroaniline [5].

⊙ based on ^{1}H NMR spectrum of 1-methylbenzimidazole [6] .

✦ based on ^{1}H NMR spectrum of 1,2-ethanediol [7].

The literature value for the chemical shifts are based on ^{1}H NMR spectra of similar compounds that model parts of binimetinib closely. The protons attached to C1, C2, and C4 are based on the ^{1}H NMR spectra of 4-bromo-2-fluoroaniline. The structure of 4-bromo-2-fluoroaniline is shown in Figure 8 next to the part of binimetinib that 4-bromo-2-fluoroaniline models.

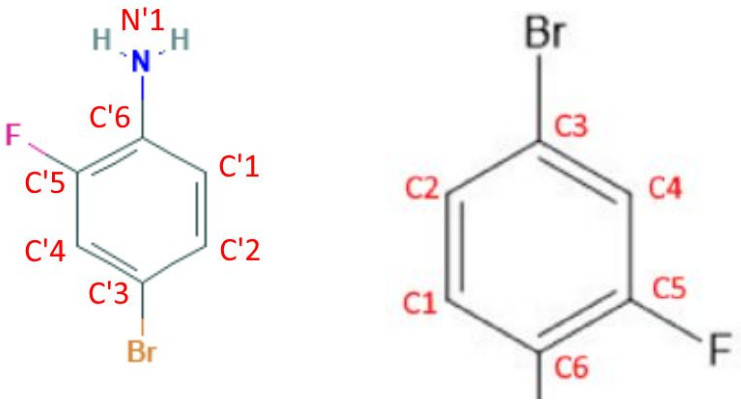

Figure 8. The structure of 4-bromo-2-fluoroaniline (left) compared to the modeled part of binimetinib (right) [8].

The ^{1}H NMR chemical shifts for the proton on C'1 differ from the chemical shift from the proton on C1 by 0.038 ppm. The chemical shifts for the proton on C'2 differ from the chemical shift from the proton on C2 by 0.22 ppm. The chemical shift difference between the proton on C'4 and C4 is 0.297 ppm. The fairly low difference in chemical shift suggests that the ^{1}H NMR spectrum determined by the HF calculation is fairly accurate.

The literature value of the chemical shifts for the protons attached to C10, C11, and C14 are based on the ^{1}H NMR spectrum for 1-methylbenzimidazole. The structure of 1-methylbenzimidazole is shown in Figure 9 next to the part of binimetinib that 1-methylbenzimidazole is modeling.

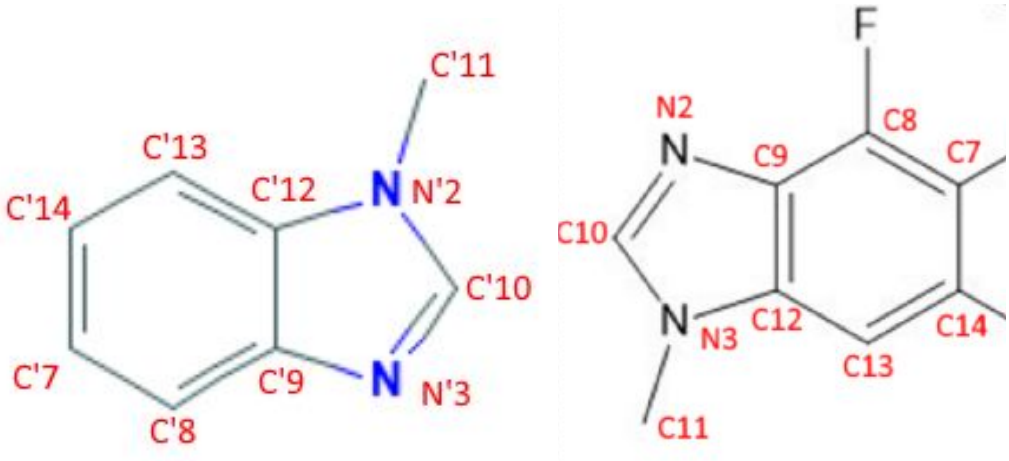

Figure 9. The structure of 1-methylbenzimidazole (left) compared to the modeled part of binimetinib (right) [9].

The calculated chemical shift for the proton on C10 is similar to the chemical shift of the proton on C'10 with a difference of 0.062 ppm. This is expected because the chemical environment around C10 is nearly identical to the chemical environment close to C'10. For the same reason, the proton on C11 is 0.125 ppm shifted more nearfield than the proton on C'11. The largest difference in chemical shift is the proton bonded to C13, which had a difference of 0.44 ppm. The small differences between the ^{1}H NMR spectrum determined by the HF calculation method and the experimentally determined ^{1}H NMR spectrum of a similar compound demonstrates the accuracy of the HF calculation method for ^{1}H NMR.

The literature values for the chemical shift of the protons on C16 and C17 is modeled by 1,2-ethanediol. The structure of 1,2-ethanediol is shown in Figure 10, below with the part of binimetinib that is modeled.

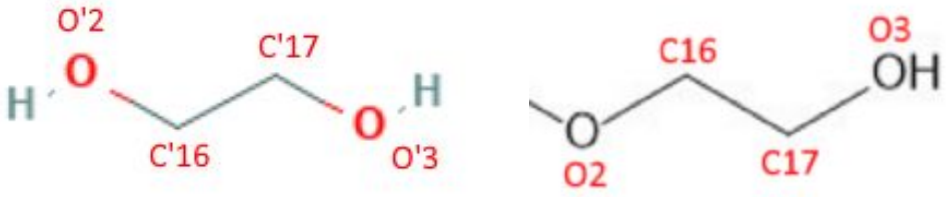

Figure 10. The structure of 1,2-ethanediol (left) compared to the part of binimetinib that it models (right) [10].

The calculated chemical shift of the proton on C16 (3.93 ppm) was higher than the chemical shift of the proton attached to C17 (3.67 ppm). This is expected because the proton on C16 is beta to an electronegative nitrogen atom, so the inductive effect causes the proton on C16 to be further downfield. The chemical shift of the proton on C16 differ by 0.214 ppm with the proton on C'16, and the chemical shift of the proton on C17 differed by 0.046 ppm with the proton on C'17. The 1,2-ethanediol model is closer for the proton on C17 compared to C16 because the chemical environment for 1,2-ethanediol and binimetinib are more similar around C17. The theoretical chemical shift of a proton beta to an oxygen atom is about 3.7 ppm [2]. The chemical shift for the protons on C16 (3.93 ppm) and C17 (3.67 ppm) are close to 3.7 ppm. The

chemical shift of the proton on O3 is not calculated by the HF method. However, the proton on O'3 (3.17 ppm) falls into the theoretical chemical shift for a hydroxyl group (2 ppm to 5 ppm).

The chemical shifts of the protons on N1 and N4 were not compared to a model. The chemical shifts of the protons attached to N1 do not fall in the theoretical chemical shift range of an amine (0.5 ppm to 5 ppm) and is higher at 5.15 ppm. This is likely due to the 2 aromatic rings bonded to N1, which tends to push the chemical shift downfield [2]. The protons bonded to N4 have a chemical shift greater than the theoretical chemical shift range for a primary amine; however, when 1 ppm is added for the proton neighboring a carbonyl and 2.5 ppm is added for being alpha to an oxygen atom, the theoretical chemical shift range becomes 4 ppm to 8.5 ppm [2]. The chemical shift of the proton on N4 then falls within this theoretical range. Since all of the chemical shifts determined by the HF calculation method fall within the expected chemical shift range, the ^{1}H NMR spectrum determined by the HF method is concluded to be accurate.

^{13}C NMR Analysis

Figure 11, below, shows the naming scheme again for each carbon atom (C#), nitrogen atom (N#), and oxygen atom (O#).

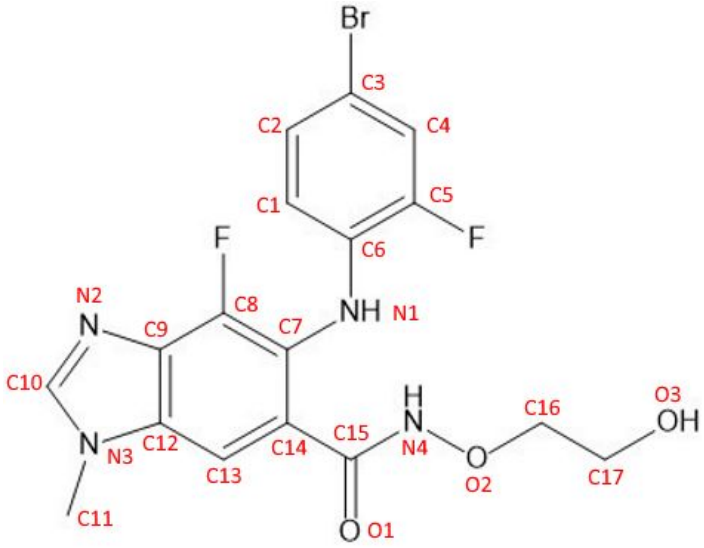

Figure 11. The structure of binimetinib with all carbon, nitrogen, and oxygen atoms labeled [1].

Figure 12, below, shows the determined ^{13}C NMR spectrum using the HF calculation method.

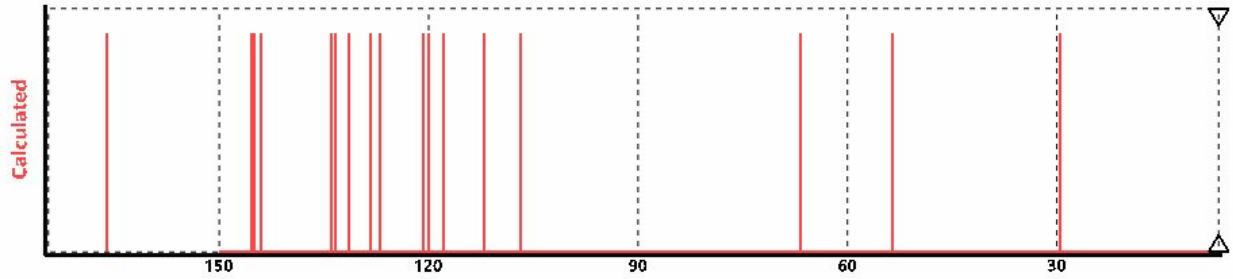

Figure 12. The ^{13}C NMR spectrum of binimetinib determined using the HF calculation method.

Figure 13 shows a closer view (between 110 ppm and 150 ppm) of the calculated ^{13}C NMR spectrum shown in Figure 12.

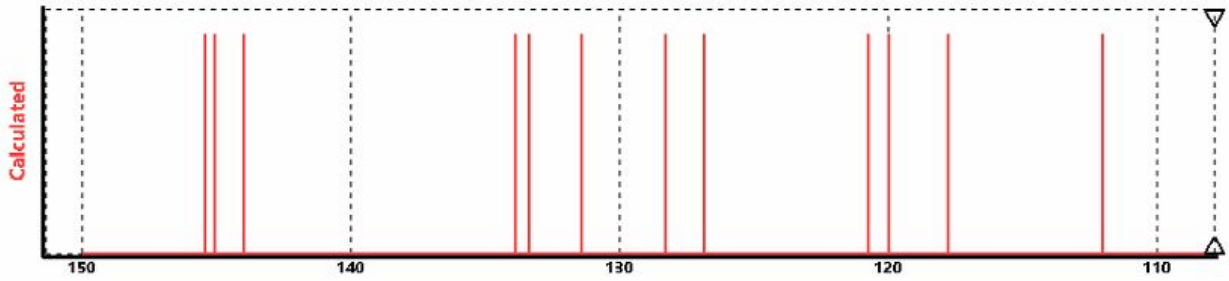

Figure 13. The ^{13}C NMR spectrum of binimetinib between 110 ppm and 150 ppm determined using the HF calculation method.

Because there are 17 carbon atoms, each in unique chemical environments, the ^{13}C NMR spectrum should produce 17 unique peaks. Based on Figure 12 and Figure 13, the HF model calculates 17 unique peaks for the ^{13}C NMR.

The majority of the peaks are between the theoretical range of 100 ppm to 150 ppm because all of the carbon atoms except for C11, C16, and C17 are sp^2 hybridized carbon atoms that are more deshielded than sp^3 hybridized carbon atoms. C5 and C8 have high chemical shifts of 144.0 ppm and 145.4 ppm because the carbon atoms are also deshielded by the electronegative fluorine atom. C10 and C12 have high chemical shifts of 145.1 ppm and 131.4 ppm, respectively, because these carbon atoms neighbor electronegative nitrogen atoms.

The chemical shifts of C16 and C17 are all within the theoretical chemical shift range of sp^3 hybridized carbon atoms that are deshielded by oxygen atoms. C16 has a chemical shift of 66.7 ppm and C17 has a lower chemical shift of 53.5 ppm. This is because C16 is also beta to an electronegative nitrogen atom in addition to the oxygen atom.

The lowest chemical shift was the methyl carbon, C11. The chemical shift of C11 is 12.5, which is within the theoretical chemical shift range of 0 ppm to 50 ppm.

Table 3 is a tabulation of the chemical shifts of each unique carbon atom determined by the HF calculation and is compared to the experimentally determined chemical shifts of carbon atoms from compounds similar to parts of binimetinib. Table 3 also contains the theoretical chemical shift range based on Klein's *Organic Chemistry*.

Carbon ID	Calculated Chemical Shift (ppm)	Literature Chemical Shift (ppm)	Theoretical Chemical Shift Range (ppm)
C1	112.0	140.10✦	100 - 150
C2	128.3	129.04✦	100 - 150
C3	120.8	122.53✦	100 - 150
C4	120.0	120.02✦	100 - 150
C5	144.0	156.23✦	100 - 150
C6	133.9	124.34✦	100 - 150
C7	117.8	124.34✦	100 - 150
C8	145.4	171.27□	100 - 150
C9	133.4	143.81●	100 - 150
C10	145.1	143.52●	100 - 150
C11	29.5	30.96●	0 - 50
C12	131.4	134.58●	100 - 150
C13	106.7	109.31●	100 - 150
C14	126.9	-	100 - 150
C15	166.0	-	150 - 220
C16	66.7	63.71◿	50 - 100
C17	53.5	63.71◿	50 - 100

Table 3. The ^{13}C NMR chemical shift determined by the HF calculation, the chemical shift, and the theoretical chemical shift for Binimetinib.

✦ based on the ^{13}C NMR spectrum of 4-bromo-2-fluoro-1-iodobenzene [11]

□ based on the ^{13}C NMR spectrum of fluorobenzene [12]

● based on the ^{13}C NMR spectrum of 1-methylbenzimidazole [5]

◿ based on the ^{13}C NMR spectrum of 1,2-ethanediol [6]

The chemical shifts of C1 to C6 are modeled by 4-bromo-2-fluoro-1-iodobenzene, which is shown in Figure 14 , below.

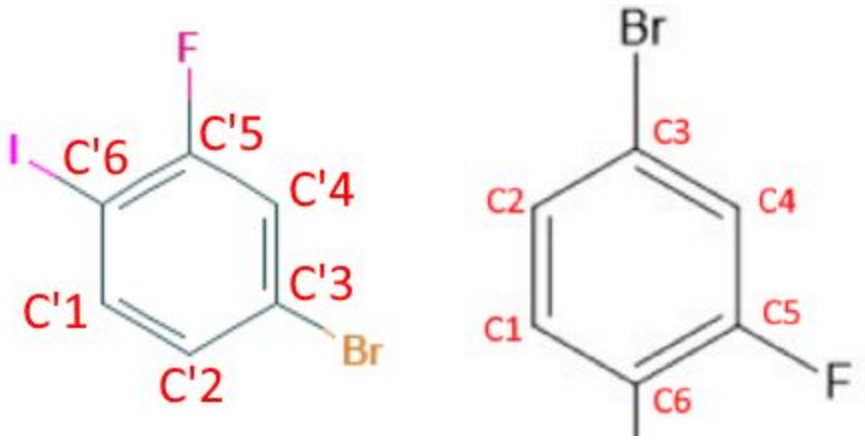

Figure 14. The structure of 4-bromo-2-fluoro-1-iodobenzene (left) compared to the modeled part of binimetinib (right) [13].

The largest difference between the experimental chemical shift and the calculated chemical shift is for C1, which has a chemical shift difference of 28.1 ppm. This is likely due to the electronegative and bulky iodine that is bonded to C'6 instead of an electronegative but smaller nitrogen bonded to C6. The difference between the chemical shifts of C6 and C'6 is 9.56 ppm, and the difference between the chemical shifts of C5 and C'5 is 12.12 ppm. The large difference is also likely caused by the presence of iodine bonded to C'6, binimetinib has a nitrogen atom bonded to C6.

The chemical shift of C8 is compared to the chemical shift of a carbon bonded to the fluorine atom in fluorobenzene. The structure of fluorobenzene is shown in Figure 15 , below.

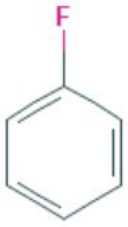

Figure 15. The bond line structure of fluorobenzene [14].

The chemical shift of C8 calculated using the HF method is 145.4 ppm, whereas the chemical shift of the carbon bonded to the fluorine in fluorobenzene is 171.27 ppm. Because binimetinib has more resonance than fluorobenzene, the chemical shift of C8 should be greater than the chemical shift of the carbon bonded to the fluorine atom in fluorobenzene; however, the opposite is observed.

The ^{13}C NMR chemical shift comparison between part of binimetinib and 1-methylbenzamide has a small chemical shift difference of about 3 ppm. See Fig. 9 for the structure of 1-methylbenzamide.

The ^{13}C NMR chemical shift comparison between part of binimetinib and 1,2-ethanediol is similar. The chemical shift of C16 differ from C'16 by 2.69 ppm and the chemical shift of C17 differed from C'17 by 10.21 ppm. Because the carbon atoms in 1,2-ethanediol are chemically equivalent, both C'16 and C'17 are experimentally determined to have a chemical shift of 63.71 ppm. However, C16 and C17 are not chemically equivalent because C16 is also beta to an electronegative atom, so it has great deshielding, resulting in a higher chemical shift.

The chemical shifts of C14 and C15 are not compared to any similar compound. Because the theoretical chemical shift of ^{13}C NMR is large, all of the chemical shifts determined by the HF calculation is within the theoretical chemical shift range. Although the ^{13}C NMR chemical shift differences are much larger than the ^{1}H NMR chemical shift differences, the ^{13}C NMR spectrum determined by the HF calculation is still fairly accurate for determining the ^{13}C NMR spectrum for binimetinib.

Conclusion

The IR spectrum determined by the HF calculation method (see Fig. 4) accurately determines the wavenumber range of the carbonyl functional group, aromatic groups, hydroxyl group, and amine groups within the theoretical wavenumber range as defined by Klein's *Organic Chemistry*. The sp^3 and sp^2 hybridized C-H bond signals are not visible in the expected theoretical wavenumber range of 2900 cm^{-1} to 3100 cm^{-1}. Two unexpected high energy signals at around 3800 cm^{-1} and 3950 cm^{-1} are also found on the IR spectrum determined by the HF calculation. A plausible explanation for the unexpected and missing peaks is that the HF calculation determined these signals to have a higher wavenumber than what is theoretically expected. Overall, the HF calculation does not produce a dependable IR spectrum for binimetinib.

The ^1H NMR spectrum determined by the HF calculation method (see Fig. 7) produces0 10 distinct proton signals. There should be 11 expected signals because there are 11 groups of protons in unique chemical environments. The missing signal is from the hydrogen bonded to an oxygen atom, O3 (see Table 2). The multiplicity for the signals determined by the HF calculation match the theoretical splitting patterns of the protons in binimetinib. The ratios of integration of the 10 signals also match the number of hydrogens that share the same chemical environment. The chemical shifts of the 10 signals determined by the HF calculation is compared to the chemical shifts of carbon atoms in similar compounds that model parts of binimetinib. The calculated signals have chemical shifts similar to the experimentally determined chemical shifts. All of the determined chemical shifts fall into the theoretically expected chemical shift range as defined by Klein's *Organic Chemistry*. The ^1H NMR determined by the HF calculation is, therefore, accurately determined for binimetinib but may not be accurate for chemicals with protons bonded to oxygen atoms..

The ^{13}C NMR spectrum determined by the HF calculation method (see Fig. 12) results in 17 distinct signals for the 17 carbon atoms in binimetinib because each carbon is in a unique chemical environment. The chemical shifts for the 17 distinct signals is compared to the chemical shifts of carbon atoms in similar compounds that model parts of binimetinib. The

chemical shifts determined by the HF calculation are mostly similar to the experimentally determined chemical shifts of similar compounds. All of the 17 signals have chemical shifts that are within the range of chemical shifts that are theoretically expected [2]. The ^{13}C NMR determined by the HF calculation is, therefore, accurately determined for binimetinib.

References

[1] Binimetinib. Focus Biomolecules. https://focusbiomolecules.com/binimetinib-mek1-2-inhibitor/ (accessed Oct 1, 2019).

[2] Klein, D. *Organic Chemistry*, 2nd ed.; John Wiley and Sons: Hoboken, 2015; pp 684-774.

[3] Savizky, R. Intro to Computational Chemistry, 2016. https://moodle.cooper.edu/moodle/course/view.php?id=133 (accessed Oct 1, 2019).

[4] National Institute of Advanced Industrial Science and Technology. Spectral Database for Organic Compounds: SDBS; https://sdbs.db.aist.go.jp/sdbs/cgi-bin/landingpage?sdbsno=898 (4-bromo-2-fluoroaniline) (accessed Mar 8, 2020).

[5] National Institute of Advanced Industrial Science and Technology. Spectral Database for Organic Compounds: SDBS; https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi (1-methylbenzimidazole) (accessed Mar 8, 2020).

[6] National Institute of Advanced Industrial Science and Technology. Spectral Database for Organic Compounds: SDBS; https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi (1,2-ethanediol) (accessed Mar 8, 2020).

[7] Starkey, L. H NMR Chemical Shifts. California State Polytechnic University https://www.cpp.edu/~lsstarkey/courses/NMR/NMRshifts1H-general.pdf (accessed Mar 8, 2020).

[8] PubChem. 4-bromo-2-fluoroaniline. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/123050 (accessed Mar 8, 2020).

[9] PubChem. 1-methylbenzimidazole. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/95890 (accessed Mar 8, 2020).

[10] PubChem. 1,2-ethanediol. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/1_2-Ethanediol (accessed Mar 8, 2020).

[11] National Institute of Advanced Industrial Science and Technology. Spectral Database for Organic Compounds: SDBS; https://sdbs.db.aist.go.jp/sdbs/cgi-bin/landingpage?sdbsno=898 (4-bromo-2-fluoro-1-iodobenzene) (accessed Mar 8, 2020).

[12] National Institute of Advanced Industrial Science and Technology. Spectral Database for Organic Compounds: SDBS; https://sdbs.db.aist.go.jp/sdbs/cgi-bin/landingpage?sdbsno=898 (fluorobenzene) (accessed Mar 8, 2020).

[13] PubChem. 4-bromo-2-fluoro-1-iodobenzene. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/2724516 (accessed Mar 8, 2020).

[14] PubChem. fluorobenzene. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/fluorobenzene (accessed Mar 8, 2020).