Synthesis, Spectroscopic Characterization, Structural Studies, Thermal and Molecular Docking of *N*-(2-Methyl-5-nitrophenyl)-4-(pyridin-2-yl)-pyrimidin-2-amine, a precursor for drug design against Chronic Myeloid Leukemia

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Abstract

The synthesis, crystal structure, spectroscopic and electronic properties of *N*-(2-methyl-5-nitrophenyl)-4-(pyridin-2-yl)-pyrimidin-2 amine, (**NPPA**), a potential template for drug design against Chronic Myelogenous Leukemia (**CML**), is reported. The design and construction of the target molecule were carried out starting from the guanidinium nitrate salt (previously synthesized) and the corresponding enamnone. X-ray diffraction analysis and the study of Hirshfeld surfaces revealed important interactions between the nitro group oxygens and hydrogen atoms of both the pyridinic and pyrimidinic rings. A crystalline ordering in layers, by stacking of rings through interactions of the $\pi \cdots \pi$ type, was observed and confirmed by the study of *Shape-index* surfaces and dispersion energy calculations. Quantitative electrostatic potential studies revealed the most positive value of the molecule on regions close to *N*—*H* group (34.8 kcal/mol), nevertheless, steric impediments and the planarity of the molecule do not allow the formation of hydrogen bonds from this group. This interaction, is however activated when the molecule takes on a new extended conformation in the active pocket of the enzyme kinase (ID **2HY**Y), interacting with protein residues that are fundamental in the inhibition process of Chronic Myeloid Leukemia (**CML**). The most negative values of the molecule are shown on regions close to the nitro group (-35.4 kcal/mol and -34.0 kcal/mol). The molecular docking study using *Vina* software revealed an energy affinity of $\Delta G = -10.3$ kcal/mol for (**NPPA**) which, despite not having a more negative value than the control molecule (Imatinib) $\Delta G = -12.8$ kcal/mol, shows great potential to be used as a template for new drugs against **CML**.

1. Introduction

Pyridine and pyrimidine rings are common building blocks in the complex structures used by nature, as well as in the synthesis of various therapeutic agents with pharmacological activity. Indeed these rings are important parts of the code of life itself, forming the core of nucleotide structures and RNA, and famously interacting by means of hydrogen bonds in order to maintain the double helix of DNA (Shakkeed *et al.*, 1989; Day *et al.*, 1973). Some examples that highlight the presence of these rings are vitamin B6 (Snell, 1958), neurotransmitters (Parra *et al.*, 2018), antivirals (Bernardino *et al.*, 2006; Chezal *et al.*, 2010) and anticancer compounds in various different lines (Al-Majid *et al.*, 2019). Among these last compounds, the family of molecules that show activity against chronic myeloid leukemia (**CML**) and whose nuclei have either a pyridine or pyrimidine, or both rings, are of particular interest here. **CML** is a myelo-proliferative disorder that results in a premature expansion of myeloid progenitor cells (starting point for the creation of red blood cells, platelets and many types of white blood cells) at different stages of maturation resulting in a shifting of the balance between self-renewal and differentiation (Strife & Clarkson, 1988). *Nilotinib* (Piccaluga *et al.*, 2012), *Dasatinib* (Foà *et al.*, 2011), *Bosutinib* (Steinbach *et al.*, 2013) and *Imatinib* (Zimmermann *et al.*, 2001) can all be used to treat **CML** and all are based around pyridine and pyrimidine rings. **CML** is associated with the presence of an aberrant chromosome, known as

the Philadelphia chromosome (Nowell *et al.*, 1960) resulting from a translocation between the long arms of chromosomes t(9,22)-(q34;q11) (Rowley, 1973) whose BCR-ABL (Guo *et al.*, 1991) fused oncogene produces a tyrosine kinase-type oncoprotein involved in cell regulation, growth and apoptosis. Knowledge of this protein led to extensive research in this field, leading to the design of the inhibitor *Imatinib* (Capdeville *et al.*, 2002), one of the most popular drugs for the treatment of CML. Despite the initial effectiveness of the drug, prolonged treatment of the disease has led to the appearance of cases of resistance (Branford & Hughes, 2006; Chandrasekhar *et al.*, 2019), which has encouraged the design of new molecules and organic nuclei to treat this disease (Zhao *et al.*, 2019). In this study, we describe an in-depth theoretical/experimental study of the NPPA nucleus at the molecular heart these anti-CML treatments, highlighting some of the structural features that confer its special active site activity and compare its structural and binding features with those of *Imatinib*.

2. Experimental

2-methyl-5-nitroaniline (1). To a balloon with sulfuric acid (7000 mg, 71.4 mmol) cooled to -10 °C (salt/ice, cold bath), o-toluidine (500 mg, 4.19 mmol) was added with vigorous stirring. An acid mixture (853 mg HNO₃ and 1650 mg H₂SO₄) was added dropwise (-10 °C) for 2 h. The mixture was put on ice and basified with NaOH. The orange precipitate was collected by filtration and washed with water. Yield (90 %), Melting point: 85-90 °C. ¹H-RMN (400 MHz, CDCl₃) δ ppm: 7.52 (dd, *J* = 8.1 Hz, 1H, Ar—H), 3.71 (br, 2H, -NH₂), 2.22 (s, 3H, -CH₃). ¹³C-RMN(100 MHz, CDCl₃) δ ppm: 147.46, 145.46, 130.82, 129.61, 113.35, 108.86, 17.68(-CH₃).

N-(2-methyl-5-nitrophenyl) guanidinium nitrate (2). To a solution of (1) (500 mg, 3.12 mmol) in propanol (4 mL), 65% nitric acid (363 mg, 3.75 mmol) was added until the formation of a yellow solid, then a 50% solution of cyanamide in water (265 mg, 6.24 mmol) was added. The reaction was refluxed for 24h and then cooled to 0 °C. The yellow precipitate was filtered off and washed with propanol and methanol. Product (54 %). Melting point: 210 °C. ¹H-RMN (400 MHz, (CD₃)₂SO) δ ppm: 8.13 (d, *J* = 8.5 Hz, 1H, Ar—H), 8.06 (s, 1H, Ar—H), 7.62 (d, *J* = 8.4 Hz, 1H, Ar—H), 7.43 (s, 1H, -NH-CNHNH₂), 2.31 (s, 1H, -CH₃). ¹³C-RMN (100 MHz, (CD₃)₂SO) δ ppm: 156.42, 146.61, 144.01, 134.62, 132.52, 122.99, 122.86, 17.82 (-CH₃).

N-(2-methyl-5-nitrophenyl)-4-(2-pyridinyl)-pyrimidin-2-amine (NPPA). To a solution of (2) (300 mg, 1.05 mmol) in n-propanol (5mL), sodium hydroxide was added and then stirred for 10 min. 3-Dimethylamino-1-(2-pyridinyl)propanone (3) (222.6 mg, 1.2 mmol) was added to the mixture and brought to reflux for 48 h, finally the mixture was brought to 0 °C. The brown precipitate was collected by filtration and washed with a mixture of propanol/methanol solution. Yield (92%), Melting point: 193 °C ¹H-RMN (400 MHz,(CD₃)₂SO) δ ppm: 9.16 (s, 1H, -NH), 8.85 (d, *J* = 2.5 Hz, 1H, Ar—H), 8.73 (d, *J* = 4.3 Hz, 1H, pirid-H), 8.65 (d, *J* = 5.1 Hz, 1H, pirimid-H), 8.34 (d, *J* = 7.8 Hz, 1H, pirid-H), 7.98 (td, *J* = 7.7, 1.8 Hz, 1H, piridi-H), 7.88 (dd, *J* = 8.4, 2.5 Hz, 1H, Ar—H), 7.77 (d, *J* = 5.0 Hz, 1H, pirimid-H), 7.54 (dd, *J* = 7.4, 4.8 Hz, 1H, pirid-H), 7.50 (d, *J* = 8.4 Hz, 1H, Ar—H), 2.42 (s, 2H, -CH₃). ¹³C-RMN (100 MHz, (CD₃)₂SO) δ ppm: 162.89, 160.25, 159.95, 153.44, 149.74, 145.95, 139.05, 138.61, 137.61, 131.34, 125.95, 121.07, 117.92, 117.28, 108.82, 18.45(-CH₃). FT—IR(cm⁻¹):3448, 3145, 3090, 3063, 1578, 1553, 1518, 1474, 1452, 1430, 1399, 1380, 1342, 1308, 1285, 1263, 1238, 1115, 1096, 1077, 1055, 989, 963, 888, 847, 825, 800, 781, 734, 699, 680, 649, 617.

2.1. Synthesis and crystallization

Synthesis

The synthetic route used to obtain NPPA, is shown in Figure 1.

Figure 1. Synthetic route to formation of NPPA.

The first stage of the synthesis corresponds to a nitration. The reaction gives rise to a nitro substituent in the *meta* position with respect to the methyl group and *para* with respect to the amino group of the o-toluidine favored by the reaction conditions (acidic medium). In this situation, the amine is able to protonate giving rise to the highly deactivating ammonium salt, with a tendency to orient in the *meta* position in electrophilic aromatic reactions (Ouellette & Rawn, 2014). Once the compound (**1**) was obtained, the synthesis of (**2**) was undertaken. Numerous reports relating to this synthesis can be found in the literature (Sugino *et al.*, 1942; Bannard *et al.*, 1958). The guanidinium salt (**2**) was synthesized using cyanamide as precursor, with acid addition at the end of this stage. In the final stage of the reaction, we proceeded with the synthesis of *N*-(2-methyl-5-nitrophenyl)-4-(2-pyridinyl)-pyrimidin-2-amine, (**NPPA**), following the methodology reported by Bredereck *et al.*, 1964 (see Experimental section for full details).

Figure 2. (a) Crystals of compound NPPA, (b) crystals upon changing the plane of polarized light with calculated morphology inset, and (c) ORTEP diagram of the contents of the asymmetric unit of the **NPPA** with non-H atoms shown as 50 % probability ellipsoids and hydrogen atoms shown as spheres of arbitrary radius.

2.2. Refinement

All non-H atoms were modelled anisotropically and the structure was refined to convergence using SHELXL (Sheldrick, 2015) as included in *WinGx* software (Farrugia, 2012). All the H atoms were found in a difference-Fourier map but were positioned at geometrically idealized positions, C—H = 0.95 Å (ring), C—H = 0.98 Å (methyl) and were refined using a riding-model approximation, with $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}(C)$ for the aromatic and methyl groups respectively (Lübben *et al.*, 2014). The H4N atom was freely and isotropically refined and gave a N—HN4 distance of 0.86 (2) Å. All bond lengths and bond angles are within normal ranges (Allen *et al.*, 1987). Table 1 gives selected crystallographic parameters, data collection and structure refinement details.

3. Results and discussion

Crystals have a light yellow tone (Fig. 2, a) and their morphology, as modelled using the hkl indices by means of *SHAPE* software (Baughman, 1991) (Fig. 2, b), was found to coincide with that observed by microscopy. Figure 2 (c) shows the molecular structure of the compound and shows that **NPPA** consists of three rings: pyridinic (**A**) (r.m.s deviation of 0.0023 Å), pyrimidinic (**B**) (r.m.s deviation of 0.0018 Å), and the C₆ aromatic ring substituted by a nitro group (**C**). These have an essentially coplanar conformation with the **A** and **C** rings inclined by 8.51 (8)° and 3.29 (8)° respectively to central ring **B**. A high degree of planarity observed between **B** and **C**, is probably aided by the formation of the non-classical intra-molecular hydrogen bond C11—H11[⋯]N2. This interaction may also favour the syn arrangement of the nitro group of ring **C** with respect to N2. The N3 nitrogen atom in the pyridine ring adopts an anti orientation with respect to N2, as a way to avoid possible interactions between the lone pairs of the nitrogen atoms.

Supramolecular analysis

The interactions of **NPPA** with its close neighbors were found using the PARST program (Nardelli, 1983). These strong and weak molecular contacts combine to give networks of intermolecular interactions, and these were found and visualised using *Mercury* software (Macrae *et al.*, 2020). The most important intra-intermolecular interactions, together with symmetry information are shown in Table 2. The crystalline packing of **NPPA** is mainly regulated by the presence of C—H[⋯]O hydrogen bonds. The C2—H2[⋯]O1ⁱ and C9—H9[⋯]O2ⁱⁱ interactions link three molecules in fused R³₂(24) rings (Etter, 1990) that stack parallel to the [1 0 -1] direction (see Table 2 and Fig. 3a). This trimeric arrangement is internally supported by C7ⁱⁱ—H7ⁱⁱ[⋯]N3 interactions between pyridine groups. Weak C16—H16[⋯]Cg2ⁱⁱⁱ interactions occur, where Cg2 is the centroid of pyridine ring **A**, with a H16[⋯]Cg2 distance of 2.97 Å. Ring stacking interactions between the **C** and **A** rings are also observed, as shown by a centroid to centroid distance of 3.743 Å (Fig. 3b).

Figure 3. a) Partial crystal structure of **NPPA**, showing the formation of $R^3_3(24)$ fused rings. These fused rings run parallel to $[101]$. **b)** Additional $C-H\cdots\pi$ and $Cg\cdots Cg$ interactions along $[1\ 0\ -1]$. Symmetry codes: (iii) $1-x, 2-y, 1-z$; (iv) $2-x, 2-y, 1-z$.

Table 2. Geometry of intra-intermolecular bonds in **NPPA** ($\text{\AA}$, $^\circ$)

Although the formation of hydrogen bonds from the lone classic hydrogen bond donor group $N-H$ would be expected, they are not observed. The explanation for this will be sought later.

Hirshfeld surface and Fingerprint analysis

Hirshfeld surfaces (**HS**) and their fingerprints were calculated to quantify the intermolecular contacts inherent in the **NPPA** structure. **HS** analysis of **NPPA** was performed using CrystalExplorer software (Mackenzie *et al.*, 2017; Spackman & Jayatilaka, 2009), an excellent tool for the exploration of non-covalent type interactions that provides information on intermolecular contacts as revealed by the proximity of the closest neighboring molecules. The distances from the Hirshfeld surface to the nearest nucleus inside, d_i , and outside the surface, d_e , are useful for defining the contact distance d_{norm} , each normalized to the van der Waals radius (vdWrad_{ii}) of each particular atom involved. In the color code adopted, red surfaces correspond to distances shorter than the vdWrad_{ii}, and white and blue surfaces indicate distances equal to or greater than the vdWrad_{ii} respectively. This three-dimensional information, and the fingerprint plot (combination of d_i and d_e) for **NPPA**, are shown in Figure 4. Not that CrystalExplorer normalises H-atom positions prior to analysis.

Figure 4. a) Hirshfeld d_{norm} surface, symmetry codes: i) $-1+x, y, -1+z$ ii) $-1/2+x, 5/2-y, -1/2+z$. **b)** fingerprint plot showing characteristic interactions. For fingerprint, blue shows ($N\cdots H/H\cdots N$) and green ($O\cdots H/H\cdots O$).

Fingerprint analysis for the **NPPA** shows that $N\cdots H/H\cdots N$, $O\cdots H/H\cdots O$ and $H\cdots H$ interactions comprise 13.4%, 15.3% and 34.5% of the total surface area respectively. Although $N\cdots H/H\cdots N$ interactions occupy a similar percentage of the Hirshfeld surface as do $O\cdots H/H\cdots O$ interactions, those involving N implicate longer distances, engaging the interactions of the NH group with O, C or N atoms very weakly. Thus, the role played by $N-H\cdots O$ interactions in related systems is seriously affected in the present study. For this reason the $O\cdots H/H\cdots O$ contributions that arise from $C-H\cdots O$ interactions and that are mostly represented in the growth by $C2-H2\cdots O1$, reaffirm the ordering that the system acquires in the crystallization process. In addition to the $C-H\cdots O$ hydrogen bonds, non-covalent intermolecular interaction involving aromatic rings can be seen and these are of a sort commonly thought to play a significant role in the properties and conformations of molecular assemblies (Meyer *et al.*, 2003). Parallel displaced $\pi-\pi$ stacking involving **A**—**B** with **C**, and pyridine **A** rings in $C-H/\pi$ interactions with methyl groups are detected (Figure 5 and Figure 3b). The supramolecular arrangement of **NPPA** shows planes at a distance of 3.342 $\text{\AA}$ and it exhibits a pattern in which, the supramolecular sheets shown in Figure 5, (a) is consistent with the crystalline planes (-404) and $(40-4)$. The $\pi-\pi$ interactions are revealed on the shape-index surface (McKinnon *et al.*, 2004) by the appearance of complementary triangles (blue and red, Figure 5 b). This interaction can be represented qualitatively and quantitatively by a fingerprint diagram, Figure 5, c) (red circle), which provides information on the $C-C$ contacts, which comprise 6.6% of the total interactions and is related to the $\pi-\pi$ stacking behavior.

Figure 5. a) Layered arrangement. **b)** Stacking $\pi-\pi$ interactions using shape index surfaces. **c)** fingerprint .

Energy Frameworks

The supramolecular study in terms of the energies involved was carried out using *CrystalExplorer* software for the treatment of intermolecular interactions (Mackenzie *et al.*, 2017). The energy is expressed in terms of E_{ele} (classical electrostatic energy), E_{rep} (repulsion energy), E_{dis} (dispersion or scattering energy) and E_{pol} (polarization energy). The interaction energies are represented as cylinders linked through centroids of pairs of molecules (electrostatic-red,

scattering-green, total energy-blue); the diameter of these cylinders being proportional to the energy involved in the interaction (Figure 6). These calculations use as a starting point, the results obtained from the X-ray diffraction experiment.

Figure 6. Diagram of energy lattices viewed from the (-101) plane of the unit cell, a) electrostatics (red), b) scattering (green), c) total energy (blue), d) total energy and scattering cylinders along the crystalline direction [010]. A scale factor of 100 was used for the width of the cylinders and energy lattices with magnitudes less than 10 kJ mol⁻¹ were omitted for a, b and c. For d, energies less than 20 kJ mol⁻¹ were omitted.

Figure 6 and Table 3 show the diagram of energy lattices and the interaction of energy of pairs of molecules calculated at a B3LYP/6-31G(*d,p*) level of theory. The results allow a quantitative evaluation of the interactions present in the crystal in terms of their nature and energy value. A 3x3 matrix of molecules (Figure 6, a) was used, taking the central molecule as a reference for the energy calculations. The study of these diagrams makes it possible to explain some of the mechanical properties of certain drugs such as tableability (ease of forming a tablet from a powder) (Turner *et al.*, 2015) and the study of polymorphs to evaluate the relationship between the structure and its properties (Tan *et al.*, 2019). From Table 3, it can be seen that the most important contributions to the total energy derive from the dispersion terms (-99.2 kJ mol⁻¹ and -95.4 kJ mol⁻¹) corresponding to the upper and lower ring stacking with respect to the central plane between molecules related by inversion centers (Figure 6, d). The contributions from electrostatic-type energies (corresponding to the in-plane interactions as opposed to the between plane ring stacking above) are less significant and could be largely cancelled out by the repulsion terms in each case. The stacking interactions dominated by dispersion energies also present considerable repulsive contributions (54.4 kJ mol⁻¹ and 56.4 kJ mol⁻¹) but these are compensated by a higher contribution to the energy of these dispersive terms. The most significant contributions are observed for interactions between molecules whose distance between centroids is less than 5 Å.

Table 3.

Electrostatic potential analysis

Molecular electrostatic potential (**MEP**) (Poltzer & Truhlar, 1981) has been a tool of quantum chemistry used by researchers to study and understand the relationships between structure and activity of molecules. It is therefore interesting to carry out a study on the behavior of the charge distribution in the **NPPA** molecule, with the aim of visualizing the possible interactions it may have with other molecules. For this purpose, the study of qualitative electrostatic potential surfaces was carried out using the *Gaussian 09W* software (Frisch *et al.*, 2009) and a quantitative analysis was performed using the *Multiwfn* software (Lu & Chen, 2012). Regions with high negative potentials (red, Figure 7 left side) are rich in negative charges and can present nucleophilic interactions. These regions are mainly located on both oxygen atoms of the nitro group and on the pyridine N atom N3. They are responsible for the most important electrostatic contacts in the crystal structure (Figure 3).

By comparing the results obtained from the analysis of Hirshfeld surfaces and electrostatic potential surfaces, it is possible to explain the reason for the interactions between O1...H2, O2...H9 and N3...H7 (Figure 3). The expected trend is to find close interactions between regions where the molecular electrostatic potential (**MEP**) is opposite. In the last mentioned interaction this behavior can be observed: the nitrogen atom in the pyridine ring N3 has a negative value of **MEP** (-21.0 Kcal/mol) and hydrogen H7 has a positive value (16.1 Kcal/mol). The maximum **MEP** are found on the oxygen atoms of the nitro group and these interact with aromatic H atoms that have values of 19.5 and 17.0 Kcal/mol. It might be expected that the nitro O atoms should interact with the N—H group which has by far the largest positive surface charge (34.8 kcal/mol) (Fig. 7, left side). However, neither this nor any other significant interactions between N—H and neighboring molecules are observed. This is possibly due, to steric impediments generated by the pyrimidinic ring and by the methyl group, and in the planarity of the molecule that does not allow it to open a gate that allow it to

activate the hydrogen bond from N—H. The effectiveness of N—H as a hydrogen bond donor is however apparent in the docking studies below.

One of the significant aspects involved in this **MEP** study lies in the explanation of the possible origin of the experimentally observed closed conformation adopted by this molecule with respect to an extended conformation. It is possible to highlight that between the atoms (H11—N2—H6—O2) in the central part of the molecule (Figure 7, right side) there is a particular arrangement of potentials that can give rise to a system analogous to a quadrupole. This behavior could favor the observed arrangement.

Figure 7. Electrostatic potential (**EP**) surfaces. **EP** maxima (blue), **EP** minima (red). Values are expressed in kcal/mol.

Theoretical/experimental vibrational analysis

The vibrational and electronic motions of a molecule provide a wealth of information in defining its structure, reactivity and stability (Cremer & Kraka, 2010). The vibrational behavior was modeled using the *VEDA 4* software (Jamróz, 2013), whose optimization was derived from the crystal structure information, with the purpose of achieving a theoretical result that was faithful to the experimental one. The theoretical results were in good agreement with the experimental ones, as a small *root-mean-square deviation* (RMSD) value (0.153 calculated with VMD) (Humphrey *et al.*, 1996) was found for the computationally optimized structure. The FT—IR spectra (experimental and theoretical) are shown in Figure 8.

Figure 8. FT—IR spectra of **NPPA**, experimental (black) and calculated (red).

By applying a scale factor of 0.9588 (Bouteiller *et al.*, 2008), a high agreement between both spectra can be observed, especially in the region below 2000 cm^{-1} (Figure 9), with the highest agreement in the region between 1250 and 2000 cm^{-1} .

Figure 9. FT—IR spectra in the 2000 and 500 cm^{-1} region.

The **NPPA** compound constitutes the core of the structure in potential Imatinib analog drugs. The study of its behavior in the infrared range, which involves almost 85% of the structure and therefore can contribute effectively to the detailed vibrational analysis. In FT—IR spectra, it is common to find bands instead of discrete vibrational signals of certain bonds. With the help of the computational software *VEDA*, it is possible to decompose such bands and establish a percentage contribution through potential energy distribution analysis (%PED) (Table 4), thereby revealing the specific vibrations that are involved in such bands and assessing their contribution in weight to the bands.

Table 4. IR Assignments and distribution of percent potential energy (%PED).

The N—H stretching vibrations for different methyl-phenyl-amines are reported in the frequency interval of $(3436 \pm 17) \text{cm}^{-1}$ (Krueger & Jan, 1970; Heacock & Marion, 1956). For **NPPA** the N—H stretching vibration is around 3448cm^{-1} a value that is in the cited range. Comparison with other molecules with similar structural characteristics, such as diphenyl-amine ($3421\text{--}3429 \text{cm}^{-1}$) (Kasai *et al.*, 1973) shows that the stretching vibration shifts to low wavenumber values, even when the nature of the substituents is changed (electro-attractor or electro-donor). This behavior could lead to rationalize that the pyrimidine ring plays an important role in shifting the N—H vibrational frequency to higher wavenumber values, causing an effect similar to that caused by a methyl group. Pyridine ring shows strong absorptions in the $1500\text{--}1600 \text{cm}^{-1}$ region caused by the stretching vibrations present in the C=C and C=N bonds. These absorptions can also be observed in the intervals: $1620\text{--}1640 \text{cm}^{-1}$, $1520\text{--}1580 \text{cm}^{-1}$, $960\text{--}1000 \text{cm}^{-1}$ and $775\text{--}825 \text{cm}^{-1}$. By way of comparison with a structurally close molecule, 4-nitrotoluene (Ahmad *et al.*, 1996), the dominant vibrational band due to the aromatic C=C stretching is found around 1598cm^{-1} , such absorptions also occur at $1575\text{--}1615 \text{cm}^{-1}$ and $1465\text{--}1520 \text{cm}^{-1}$. Calculated values show good agreement, occurring around 1578 , 1553 , 1399cm^{-1} . Comparing the C—H stretching vibrations of the methyl group in the N4-bonded molecule (Fig. 2, c) with the 4-nitrotoluene, it shows that the C—H

stretching vibrations are found at 3110, 3060 and 3077 cm^{-1} (Socrates, 2007). In comparison with C—H stretching vibrations for pyrimidinic compounds, these appear in the range 3000–3100 cm^{-1} (Thilagavathi & Arivazhagan, 2011; Kartha, 1982). Infrared spectroscopy studies for the 2-aminopicoline molecule show that the C—H stretching vibration is found around 3135 and 3059 cm^{-1} (Nair *et al.*, 2010). For compound NPPA, the aromatic C—H stretching vibrations are found at 3145, 3090 and 3063 cm^{-1} . At 734 cm^{-1} is the out-of-plane stress vibration, which is characterized as being present around 650–900 cm^{-1} (Bouteiller *et al.*, 2008). Asymmetric and symmetric stretching vibrations of the N—O bond in various aromatic compounds occur in the interval (1517 $\pm$ 12) cm^{-1} and (1350 $\pm$ 12) cm^{-1} respectively (Brown, 1955). For the **NPPA** compound the experimental wavenumber values for such vibrations are 1518 cm^{-1} and 1342 cm^{-1} . For the specific case of nitrobenzene, these vibrations can be found at 1523 cm^{-1} and 1347 cm^{-1} respectively. For a better approximation, the values can be compared with the 4-nitrotoluene molecule (Ahmad *et al.*, 1996), whose values are at 1517 cm^{-1} and 1344 cm^{-1} , being very close to those found in **NPPA**. The same authors reported by computational studies, B3LYP/6-311+G (d,p), asymmetric and symmetric stretching values at 1524 cm^{-1} and 1347 cm^{-1} (Clarkson & Ewen Smith, 2003); the theoretical values of these same vibrations for the **NPPA** are at 1575 cm^{-1} and 1367 cm^{-1} . The experimental value for the ONO scissor movement for **NPPA** is at 825 cm^{-1} , while the theoretical value was 846 cm^{-1} . (Table 3).

UV/Vis spectroscopy analysis

In order to identify the nature of the electronic transitions in the molecule, a UV/Vis spectroscopy study was carried out with solvents of different polarities. Figure 10 shows the electronic spectra, presenting absorption bands around 214, 249, 279 and 337 nm. It was possible to identify a "red shift", for the most energetic transition when the polarity of the solvent increases gradually (cyclohexane 209.56 nm; chloroform 212.56 nm and 1-propanol 214.02 nm). This red shift is associated with a π - π^* transition with a more polar excited state that is easily stabilized by polar solvents (Zakerhamidi *et al.*, 2012). This effect can also be associated with intramolecular charge transfer processes due to the presence of donor-acceptor groups as is also observed in the case of the p-nitroaniline molecule (Li *et al.*, 2014; Sok *et al.*, 2011).

Figure 10. UV-Vis spectra for **NPPA** in different solvents. Vertical bars show the theoretical TD-DFT behavior.

For a correct identification of the transitions, theoretical calculations were performed with the *Gaussian 09W* software, using the time-dependent density functional theory, TD-DFT (Adamo & Jackquemin, 2013) at the 6311G++(d,p) level. A value of 3.033 eV was determined for the HOMO-LUMO transition, correlated with the low intensity band observed by UV-Vis spectroscopy at 340 nm (Figure 10). This value is within the average of values found for different types of organic semiconductors (Kim & Kymissis, 2017) and shows the feasibility with which this transition can occur. By means of the NBO (Natural Bond Orbital) (Weinhold & Landis, 2001) analysis, it was able to establish the electronic density distribution and thus associate the type of transition that occurs. It was possible to determine that the electronic transition that presents a red shift (Figure 10), has a high electronic density in p_z -type orbitals with a high participation of C10 and C11 carbons (Figure 2, c) with a participation of 50.39% (p_z ; 99.97%) and 49.61% (p_z ; 99.94%). The geometry of the orbitals made possible the observation of a bonding-antibonding behavior in this transition whose dipole moment value in the excited state is 6.42 D (which explains the higher stability of this transition in polar solvents) compared to the value obtained in the ground state 3.51 D, allowing to associate to a π - π^* transition.

Thermo-gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). The results of the thermal analysis are shown in Figure 11. The study was performed in order to identify possible crystalline phase changes, and to evaluate the stability of the synthesized molecule.

Figure 11. Thermogravimetric (green) and differential calorimetry (blue) analysis for NPPA. The images taken at the indicated points are shown at the top. At point A images both without polarized light and with polarized light are shown.

Figure 11 shows relative stability from points A to B. When a temperature of approximately 170° C is reached, a change in (B) begins to be observed. Immediately after (193.18° C), **(1)** a change in the heat flux of 124.42J/g (38.2kJ/mol) is recorded, indicating a phase change, which here is the melting point of **NPPA**. At 304.56° C, **(2)** there is the beginning of a process of 19.54J/g (6kJ/mol) and immediately another process with an energy flux of 116.14J/g (36kJ/mol), associated with the thermal decomposition of the compound, followed by a second mass loss at 417.76° C, **(3)** with a heat flux of 763.18J/g (281.79kJ/mol) where the organic material is completely decomposed

Molecular docking analysis

Molecular docking studies were performed using *Vina* (Trott & Olson, 2010) computational software and *AutoDock tools*. **NPPA** was studied in the active site of the human ABL kinase (PDB ID:2HYY) which has a resolution of 2.5 Å (Cowan-Jacob *et al.*, 2006). A direct comparison was made between the co-crystallized Imatinib (STI571-Glivec) molecule and the docking result for **NPPA** to understand the effect of their structural differences (nitrogen position in the pyridine ring and presence of the nitro group). The Imatinib molecule exhibits four major hydrogen interactions at the active site. The molecular docking calculation (Figure 12) shows that **NPPA** behaves well in the active site, adopting the same conformation as the corresponding moiety in the Imatinib structure. The molecular coupling images were generated using the programs *PyMOL* and *Ligplot+* (Wallace *et al.*, 1995).

Figure 12. Co-crystallized Imatinib (cyan) and **NPPA** (magenta) at the active site of tyrosine kinase. The dashed lines with yellow indicate interactions with amino acids.

Figure 13 shows a two-dimensional comparison achieved with the *LigPlot+* program of both molecules in the active site. On the left side of the figure, the interactions of the **NPPA** (red circles and dots) at the active site, are highlighted (leaving the Imatinib and its neighboring interactions in gray). In turn, on the right side Imatinib is clearly seen, while leaving **NPPA** in gray. The calculations reveal an interaction free energy of $\Delta G = -12.8$ kcal/mol for the protein co-crystallized with Imatinib and $\Delta G = -10.3$ kcal/mol, for the protein with **NPPA**, which adopts an extended conformation. These results behave as expected, since, in the first instance, we observe the absence of interaction between the carboxylate of the glutamate residue and the oxygens of the nitro group (an interaction that is present in the Imatinib molecule, between the nitrogen of the amido group and the glutamate, Figure 13) and in second instance, by the absence of interaction between the methionine residue and the nitrogen of the pyridine ring in the **NPPA**. It is important to note the relevance of the position of the nitrogen atom *N* of the pyridine ring in Imatinib, which in its native position achieves more effectively an interaction by hydrogen bonding with the (*N—H*) present in the peptide bond of methionine (Met318) (Table 5). An important fact to highlight in **NPPA** docking is the formation of hydrogen bonds in the active site of the tyrosine kinase with the most electropositive and electronegative sites of **NPPA** (Figure 7 and Figure 13). It is possible to observe that one of the nitro group oxygens (**MEP**: -34.0 or -35.4 kcal/mol) is bound to Asp381 (Figure 13). Additionally, the most electropositive (*N—H*) site is bound to Thr315, which gives a direct involvement of the sites with the most important electrostatic potential values in **NPPA** with the key amino acids of the active site.

Figure 13. Map of **NPPA** interactions at the active site.

The structural study of **NPPA**, besides showing structural particularities present in the molecule, made it possible to introduce and make use of these parameters (hydrogen atoms in the crystalline systems, study of Hirshfeld surfaces, electrostatic potential and energetic frameworks) to explain the potential properties of this compound as a drug and its action on the active site of the human ABL kinase (PDB ID: 2HYY).

Table 5. The most relevant hydrogen bonds of the amino acid residues of 2HYY with imatinib and **NPPA**.

Conclusions It was possible to synthesize a compound, with structural characteristics similar to Imatinib, whose crystallographic studies showed a molecular arrangement in layers. The above behavior has its origin in the almost planar

structure of the molecule, with overlapping of aromatic rings evidenced in the "*shape Index*" models and in the high dispersion energies found in studies of energy frameworks. The theoretical model showed a good agreement with the electrostatic potential surfaces and supramolecular interactions of the crystalline system, where the oxygen atoms of the nitro group (more negative electrostatic potentials) participate actively in the intramolecular network. The region close to N4—H4N, which shows the highest **MEP**, does not participate in the formation of hydrogen bonds due to steric impediments and the planarity of the molecule. However, in docking studies when the title molecule is interacting in an active pocket, with an extended structural conformation, this bond is activated by forming interactions with protein residues, specifically with Th315, an essential interaction that occurs in the presence of the control drug imatinib. The molecular docking study revealed an affinity energy of $\Delta G = -10.3$ kcal/mol for **NPPA** which despite not having a more negative value than the control molecule (Imatinib) $\Delta G = -12.8$ kcal/mol, provides interesting elements for its proposal as a building block for future drug syntheses against Chronic Myeloid Leukemia.

Table 1

Experimental details

Crystal data	
Chemical formula	C ₁₆ H ₁₃ N ₅ O ₂
<i>M</i> _r	307.31
Crystal system, space group	Monoclinic, <i>P</i> 2 ₁ / <i>n</i>
Temperature (K)	123
<i>a</i> , <i>b</i> , <i>c</i> (Å)	8.0616 (5), 17.8873 (8), 9.5504 (4)
β (°)	95.490 (4)
<i>V</i> (Å ³)	1370.85 (12)
<i>Z</i>	4
Radiation type	Cu <i>K</i> α
μ (mm ⁻¹)	0.85
Crystal size (mm)	0.45 × 0.20 × 0.10
Data collection	
Diffractometer	Oxford Diffraction Gemini S diffractometer
Absorption correction	Multi-scan <i>CrysAlis PRO</i> , Agilent Technologies, Version 1.171.34.46 (release 25-11-2010 CrysAlis171 .NET) (compiled Nov 25 2010,17:55:46) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
<i>T</i> _{min} , <i>T</i> _{max}	0.687, 1.000
No. of measured, independent and observed [<i>I</i> > 2σ(<i>I</i>)] reflections	4948, 2684, 2326
<i>R</i> _{int}	0.020
(sin θ/λ) _{max} (Å ⁻¹)	0.621
Refinement	
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.043, 0.123, 1.01
No. of reflections	2684
No. of parameters	213
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement

$\Delta\rho_{\max}, \Delta\rho_{\min}$ (e Å⁻³) 0.28, -0.22

Computer programs: *CrysAlis PRO*, Oxford Diffraction Ltd., Version 1.171.34.40 (release 27-08-2010 CrysAlis171 .NET), *CrysAlis PRO*, *SIR 92* (Altomare *et al.*, 1994), *SHELXL2014/7* (Sheldrick, 2015), *Mercury* (Macrae *et al.*, 2020), *publCIF* 1.9.21_c (Westrip, 2010).

Table 2

Hydrogen-bond geometry (Å, °) for (shelx)

<i>D</i> —H··· <i>A</i>	<i>D</i> —H	H··· <i>A</i>	<i>D</i> ··· <i>A</i>	<i>D</i> —H··· <i>A</i>
C2—H2···O1 ⁱ	0.95	2.59	3.3777 (19)	140
C9—H9···O2 ⁱⁱ	0.95	2.51	3.1565 (19)	125
C11—H11···N2	0.95	2.24	2.8723 (19)	124

Symmetry codes: (i) $x-1, y, z-1$; (ii) $x-1/2, -y+5/2, z-1/2$.

Table 3

Interaction energies of pairs of molecules (kJ/mol) obtained from energy lattice calculations for the NPPA.

colour	N	Symmetry code	R	Electron density	E(El)	E(pol)	E(dis)	E(rep)	E(tot)
i	2	$-x+1/2, y+1/2, -z+1/2$	13.04	B3LYP/6-31G(d,p)	0.2	0.0	-1.1	0.0	-0.8
ii	1	$-x, -y, -z$	18.15	B3LYP/6-31G(d,p)	0.0	0.0	0.1	0.0	-0.1
iii	1	$-x, -y, -z$	15.22	B3LYP/6-31G(d,p)	0.0	0.0	-0.3	0.0	-0.2
iv	2	$x+1/2, -y+1/2, z+1/2$	10.51	B3LYP/6-31G(d,p)	-14.3	-4.1	-20	23.5	-21.0
v	2	x, y, z	11.89	B3LYP/6-31G(d,p)	-4.9	-1.4	-5.8	5.1	-8.2
vi	2	x, y, z	17.89	B3LYP/6-31G(d,p)	0.2	0.0	-0.1	0.0	0.1
vii	1	$-x, -y, -z$	10.54	B3LYP/6-31G(d,p)	-0.9	-0.2	-8.7	2.3	-7.3
viii	2	$-x+1/2, y+1/2, -z+1/2$	10.35	B3LYP/6-31G(d,p)	-6.8	-1.6	-20.1	15.5	-16.3
ix	1	$-x, -y, -z$	18.00	B3LYP/6-31G(d,p)	0.0	0.0	-0.1	0.0	-0.1
x	2	$x+1/2, -y+1/2, z+1/2$	10.97	B3LYP/6-31G(d,p)	-4.1	-2.4	-18.4	13.2	-14.0
xi	2	$-x+1/2, y+1/2, -z+1/2$	12.31	B3LYP/6-31G(d,p)	-2.4	-0.2	-1.2	0.0	-3.7
xii	1	$-x, -y, -z$	3.74	B3LYP/6-31G(d,p)	-7.8	-2.8	-99.2	54.4	-63.1
xiii	1	$-x, -y, -z$	4.39	B3LYP/6-31G(d,p)	-13.8	-2.3	-95.4	56.4	-64.6
xiv	2	$-x+1/2, y+1/2, -z+1/2$	9.96	B3LYP/6-31G(d,p)	-5.3	-0.9	-18.2	11.4	-15.1
xv	1	$-x, -y, -z$	9.48	B3LYP/6-31G(d,p)	-5.4	-2.3	-16.1	8.3	-16.4
xvi	1	$-x, -y, -z$	18.69	B3LYP/6-31G(d,p)	0.4	0.0	-0.1	0.0	0.3
xvii	1	$-x, -y, -z$	18.54	B3LYP/6-31G(d,p)	0.4	0.0	-0.1	0.0	0.3

xviii	1	-x,-y,-z	14.14	B3LYP/6-31G (d,p) _{1.3}	-0.1	-0.4	0.0	1.0
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* Scaling factors used for the determination of the total energy ETot: Eele = 1.057, Epol = 0.740, Edis = 0.871 and Erep = 0.618, R is the distance between molecule centroids (Å), N is the number of pairs of molecules in the cluster with that particular interaction energy.

Table 4

IR Assignments and distribution of percent potential energy (%PED)

Assignments (PED%)	Exp (cm ⁻¹)	6311G++(d,p) (cm ⁻¹) Without sc.	Sc.
ν NH (100)	3448	3631	3481
ν CH (99)	3145	3288	3152
ν CH (91)	3090	3115	2986
ν CH (100)	3063	3054	2928
ν CC (55)	1578	1655	1587
ν CC (14) + ν NC (12)	1553	1619	1553
ν _{as} ON (59)	1518	1575	1510
ν ON (16) + β HCC (22)	1474	1557	1492
β HCN(56)	1452	1514	1452
β HCC(42) + β HCH(78)+ τ HCCC(15)	1430	1491	1429
ν NC(43)	1399	1459	1399
β HCN(42)	1380	1435	1376
ν _s ON (64)	1342	1367	1310
ν CC (12)	1308	1338	1283
ν NC (37) + β HCN(19)	1285	1322	1267
ν NC (30) + ν CC(10)	1263	1280	1227
ν NC (34) + β HCN(10)	1238	1264	1212
β CNC(51)	1115	1130	1083
ν CC (30) + β HCC (50)	1096	1119	1073
ν CC (33) + β HCH (11)	1077	1092	1047
ν CC (45)	1055	1065	1021
ν CC (21) + β CCC (45)	989	1012	970
ν NC (10) + τ HCCC (13) + β CCC(17)	963	981	941
τ HCCC(81)	888	924	886
τ HCNC(76)	847	880	843
δ ONO(43) + ν ON(11)	825	846	811
τ HCCC(79)	800	831	797
τ HCCC(70)	781	819	785
β CNC (52) + ν CC(11)	742	768	736
τ HCCC (10) + τ HCCN(69)	734	760	729
γ CCNC(70)	649	687	659
β CCC(68)	617	632	606

ν -Stretching; β -in-plane deformation; δ -scissors; γ -out-of-plane deformation; τ -torsion; s-symmetrical; as: asymmetrical; r -rocking; t -twisting.

Table 5

The most relevant hydrogen bonds formed by the protein residues of 2HYY with imatinib and NPPA.

Imatinib					NPPA		
Residue	Amino acid	H-A (Å)	D-A (Å)	Donor angle	H-A (Å)	D-A (Å)	Donor angle
286C	Glu	2.24	3.09	143.11			
315C	Thr	2.34	3.01	124.55	2.46	3.19	143.37
318C	Met	2.00	2.94	158.24			

Acknowledgements

RM—F thanks Universidad del Valle and Banco de la República, Colombia for partial financial support—Project number CI 1169. KAD thanks Universidad del Valle for the support of his doctoral studies.

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supporting information

Synthesis, Spectroscopic Characterization, Structural Studies, Thermal and Molecular Docking of *N*-(2-Methyl-5-nitrophenyl)-4-(pyridin-2-yl)-pyrimidin-2-amine, a precursor for drug design against Chronic Myeloid Leukemia

Rodolfo Moreno-Fuquen,* Kevin Arango-Daraviña and Alan R. Kennedy

Computing details

Data collection: *CrysAlis PRO*, Oxford Diffraction Ltd., Version 1.171.34.40 (release 27-08-2010 CrysAlis171 .NET); cell refinement: *CrysAlis PRO*; data reduction: *CrysAlis PRO*; program(s) used to solve structure: *SIR 92* (Altomare *et al.*, 1994); program(s) used to refine structure: *SHELXL2014/7* (Sheldrick, 2015); molecular graphics: *Mercury* (Macrae *et al.*, 2020); software used to prepare material for publication: *publCIF* 1.9.21_c (Westrip, 2010).

(shelx)

Crystal data

$C_{16}H_{13}N_5O_2$
 $M_r = 307.31$
 Monoclinic, $P2_1/n$
 $a = 8.0616$ (5) Å
 $b = 17.8873$ (8) Å
 $c = 9.5504$ (4) Å
 $\beta = 95.490$ (4)°
 $V = 1370.85$ (12) Å³
 $Z = 4$

$F(000) = 640$
 $D_x = 1.489$ Mg m⁻³
 Cu $K\alpha$ radiation, $\lambda = 1.54184$ Å
 Cell parameters from 4950 reflections
 $\theta = 5.0$ – 73.1 °
 $\mu = 0.85$ mm⁻¹
 $T = 123$ K
 Block, brown
 $0.45 \times 0.20 \times 0.10$ mm

Data collection

Oxford Diffraction Gemini S
 diffractometer
 Radiation source: fine-focus sealed tube
 Graphite monochromator
 ω scans

Absorption correction: multi-scan
CrysAlis PRO, Agilent Technologies, Version 1.171.34.46 (release 25-11-2010 CrysAlis171 .NET) (compiled Nov 25 2010, 17:55:46) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
 $T_{\min} = 0.687$, $T_{\max} = 1.000$
 4948 measured reflections
 2684 independent reflections
 2326 reflections with $I > 2\sigma(I)$
 $R_{\text{int}} = 0.020$
 $\theta_{\max} = 73.1$ °, $\theta_{\min} = 5.0$ °
 $h = -7 \rightarrow 9$
 $k = -18 \rightarrow 21$
 $l = -11 \rightarrow 11$

Refinement

Refinement on F^2
 Least-squares matrix: full
 $R[F^2 > 2\sigma(F^2)] = 0.043$

$wR(F^2) = 0.123$
 $S = 1.01$
 2684 reflections

213 parameters
 0 restraints
 Primary atom site location: structure-invariant direct methods
 Secondary atom site location: difference Fourier map
 Hydrogen site location: mixed

H atoms treated by a mixture of independent and constrained refinement
 $w = 1/[\sigma^2(F_o^2) + (0.0719P)^2 + 0.3901P]$
 where $P = (F_o^2 + 2F_c^2)/3$
 $(\Delta/\sigma)_{\max} = 0.001$
 $\Delta\rho_{\max} = 0.28 \text{ e } \text{\AA}^{-3}$
 $\Delta\rho_{\min} = -0.22 \text{ e } \text{\AA}^{-3}$

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($\text{\AA}^2$)

	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{iso} */ <i>U</i> _{eq}
O1	1.16103 (14)	1.01117 (7)	0.92354 (12)	0.0339 (3)
O2	0.99304 (18)	1.07275 (7)	0.77704 (15)	0.0469 (4)
N1	0.51884 (16)	0.91344 (7)	0.27391 (14)	0.0287 (3)
N2	0.67099 (15)	1.00859 (7)	0.40847 (13)	0.0242 (3)
N3	0.59142 (16)	1.19155 (7)	0.25969 (13)	0.0267 (3)
N4	0.69772 (17)	0.88107 (7)	0.46299 (14)	0.0274 (3)
N5	1.05088 (17)	1.01392 (7)	0.82592 (15)	0.0293 (3)
C1	0.62803 (18)	0.93787 (9)	0.37969 (15)	0.0247 (3)
C2	0.4500 (2)	0.96675 (9)	0.19020 (17)	0.0304 (3)
H2	0.3732	0.9523	0.1133	0.036*
C3	0.48455 (19)	1.04235 (9)	0.20924 (16)	0.0284 (3)
H3	0.4331	1.0793	0.1482	0.034*
C4	0.59806 (18)	1.06101 (8)	0.32205 (15)	0.0242 (3)
C5	0.64673 (18)	1.14002 (8)	0.35555 (15)	0.0242 (3)
C6	0.74363 (18)	1.15826 (9)	0.47974 (15)	0.0264 (3)
H6	0.7798	1.1205	0.5457	0.032*
C7	0.78625 (19)	1.23231 (9)	0.50530 (16)	0.0286 (3)
H7	0.8525	1.2462	0.5889	0.034*
C8	0.73079 (19)	1.28577 (9)	0.40709 (17)	0.0285 (3)
H8	0.7580	1.3370	0.4216	0.034*
C9	0.6348 (2)	1.26264 (9)	0.28737 (16)	0.0282 (3)
H9	0.5971	1.2996	0.2203	0.034*
C10	0.80883 (18)	0.88211 (8)	0.58471 (15)	0.0240 (3)
C11	0.87791 (18)	0.94771 (8)	0.64248 (16)	0.0256 (3)
H11	0.8531	0.9945	0.5984	0.031*
C12	0.98348 (18)	0.94365 (8)	0.76537 (16)	0.0252 (3)
C13	1.02617 (19)	0.87739 (9)	0.83338 (16)	0.0277 (3)
H13	1.0991	0.8763	0.9176	0.033*
C14	0.9580 (2)	0.81246 (8)	0.77344 (16)	0.0273 (3)
H14	0.9860	0.7660	0.8178	0.033*
C15	0.85026 (18)	0.81275 (8)	0.65066 (16)	0.0250 (3)
C16	0.7805 (2)	0.74006 (8)	0.59130 (17)	0.0293 (3)
H161	0.8189	0.6989	0.6540	0.044*
H162	0.8188	0.7318	0.4982	0.044*
H163	0.6585	0.7421	0.5831	0.044*

H4N	0.660 (2)	0.8382 (12)	0.435 (2)	0.031 (5)*
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Atomic displacement parameters ($\text{\AA}^2$)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0339 (6)	0.0326 (6)	0.0330 (6)	−0.0025 (5)	−0.0075 (5)	−0.0019 (5)
O2	0.0614 (8)	0.0200 (6)	0.0536 (8)	−0.0030 (6)	−0.0236 (6)	0.0029 (5)
N1	0.0318 (7)	0.0229 (7)	0.0306 (7)	−0.0008 (5)	−0.0018 (5)	−0.0026 (5)
N2	0.0276 (6)	0.0199 (6)	0.0248 (6)	0.0002 (5)	0.0006 (5)	0.0001 (5)
N3	0.0315 (6)	0.0217 (6)	0.0265 (6)	0.0032 (5)	0.0004 (5)	0.0017 (5)
N4	0.0343 (7)	0.0159 (6)	0.0308 (7)	−0.0024 (5)	−0.0036 (5)	−0.0014 (5)
N5	0.0331 (7)	0.0229 (7)	0.0310 (7)	−0.0021 (5)	−0.0014 (5)	0.0006 (5)
C1	0.0265 (7)	0.0225 (7)	0.0249 (7)	0.0012 (5)	0.0021 (5)	−0.0013 (5)
C2	0.0314 (8)	0.0294 (8)	0.0289 (8)	−0.0007 (6)	−0.0048 (6)	−0.0037 (6)
C3	0.0316 (8)	0.0255 (8)	0.0273 (7)	0.0041 (6)	−0.0014 (6)	0.0020 (6)
C4	0.0265 (7)	0.0215 (7)	0.0251 (7)	0.0014 (6)	0.0045 (6)	−0.0002 (5)
C5	0.0256 (7)	0.0213 (7)	0.0261 (7)	0.0027 (5)	0.0045 (6)	0.0016 (5)
C6	0.0302 (7)	0.0225 (7)	0.0263 (7)	0.0024 (6)	0.0011 (6)	0.0030 (6)
C7	0.0297 (7)	0.0275 (8)	0.0279 (7)	0.0002 (6)	−0.0012 (6)	−0.0023 (6)
C8	0.0324 (8)	0.0192 (7)	0.0344 (8)	0.0001 (6)	0.0051 (6)	−0.0013 (6)
C9	0.0339 (8)	0.0207 (7)	0.0298 (8)	0.0037 (6)	0.0024 (6)	0.0049 (6)
C10	0.0254 (7)	0.0206 (7)	0.0259 (7)	0.0008 (5)	0.0033 (6)	0.0005 (5)
C11	0.0290 (7)	0.0186 (7)	0.0288 (7)	0.0011 (6)	0.0006 (6)	0.0032 (5)
C12	0.0266 (7)	0.0202 (7)	0.0288 (7)	−0.0007 (6)	0.0021 (6)	−0.0001 (6)
C13	0.0308 (7)	0.0257 (8)	0.0263 (7)	0.0011 (6)	0.0002 (6)	0.0030 (6)
C14	0.0335 (8)	0.0206 (7)	0.0281 (7)	0.0028 (6)	0.0041 (6)	0.0046 (5)
C15	0.0286 (7)	0.0187 (7)	0.0286 (7)	0.0011 (5)	0.0073 (6)	0.0011 (5)
C16	0.0392 (8)	0.0185 (7)	0.0302 (8)	0.0000 (6)	0.0035 (6)	0.0012 (6)

Geometric parameters ($\text{\AA}$, $^\circ$) for (shelx)

O1—N5	1.2255 (18)	C6—C7	1.384 (2)
O2—N5	1.2252 (18)	C6—H6	0.9500
N1—C2	1.331 (2)	C7—C8	1.383 (2)
N1—C1	1.348 (2)	C7—H7	0.9500
N2—N2	0.000 (2)	C8—C9	1.381 (2)
N2—C1	1.3333 (19)	C8—H8	0.9500
N2—C4	1.3466 (19)	C9—H9	0.9500
N3—C9	1.338 (2)	C10—C11	1.390 (2)
N3—C5	1.3448 (19)	C10—C15	1.417 (2)
N4—C1	1.377 (2)	C11—C12	1.384 (2)
N4—C10	1.398 (2)	C11—H11	0.9500
N4—H4N	0.86 (2)	C12—C13	1.379 (2)
N5—C12	1.4661 (19)	C13—C14	1.385 (2)
C1—N2	1.3333 (19)	C13—H13	0.9500
C2—C3	1.389 (2)	C14—C15	1.391 (2)
C2—H2	0.9500	C14—H14	0.9500
C3—C4	1.386 (2)	C15—C16	1.506 (2)
C3—H3	0.9500	C16—H161	0.9800
C4—N2	1.3466 (19)	C16—H162	0.9800
C4—C5	1.493 (2)	C16—H163	0.9800

C5—C6	1.395 (2)		
C2—N1—C1	115.01 (13)	C9—C8—C7	118.25 (14)
C1—N2—C4	116.41 (13)	C9—C8—H8	120.9
C9—N3—C5	116.97 (13)	C7—C8—H8	120.9
C1—N4—C10	131.63 (13)	N3—C9—C8	124.27 (14)
C1—N4—H4N	111.4 (13)	N3—C9—H9	117.9
C10—N4—H4N	116.9 (13)	C8—C9—H9	117.9
O2—N5—O1	123.09 (13)	C11—C10—N4	122.80 (13)
O2—N5—C12	118.23 (13)	C11—C10—C15	119.62 (14)
O1—N5—C12	118.68 (13)	N4—C10—C15	117.58 (13)
N2—C1—N1	126.89 (14)	C12—C11—C10	118.87 (14)
N2—C1—N4	119.83 (13)	C12—C11—H11	120.6
N1—C1—N4	113.27 (13)	C10—C11—H11	120.6
N1—C2—C3	123.51 (14)	C13—C12—C11	123.35 (14)
N1—C2—H2	118.2	C13—C12—N5	118.99 (14)
C3—C2—H2	118.2	C11—C12—N5	117.65 (13)
C4—C3—C2	116.44 (14)	C12—C13—C14	117.05 (14)
C4—C3—H3	121.8	C12—C13—H13	121.5
C2—C3—H3	121.8	C14—C13—H13	121.5
N2—C4—C3	121.73 (14)	C13—C14—C15	122.44 (14)
N2—C4—C5	115.94 (13)	C13—C14—H14	118.8
C3—C4—C5	122.34 (14)	C15—C14—H14	118.8
N3—C5—C6	122.73 (14)	C14—C15—C10	118.66 (14)
N3—C5—C4	116.08 (13)	C14—C15—C16	119.66 (13)
C6—C5—C4	121.19 (14)	C10—C15—C16	121.68 (14)
C7—C6—C5	118.88 (14)	C15—C16—H161	109.5
C7—C6—H6	120.6	C15—C16—H162	109.5
C5—C6—H6	120.6	H161—C16—H162	109.5
C8—C7—C6	118.89 (14)	C15—C16—H163	109.5
C8—C7—H7	120.6	H161—C16—H163	109.5
C6—C7—H7	120.6	H162—C16—H163	109.5
C4—N2—C1—N2	0 (100)	N2—C4—C5—C6	−8.5 (2)
N2—N2—C1—N1	0.0 (3)	C3—C4—C5—C6	171.36 (14)
C4—N2—C1—N1	−0.4 (2)	N3—C5—C6—C7	−0.6 (2)
N2—N2—C1—N4	0.0 (3)	C4—C5—C6—C7	179.57 (13)
C4—N2—C1—N4	179.78 (13)	C5—C6—C7—C8	0.2 (2)
C2—N1—C1—N2	0.8 (2)	C6—C7—C8—C9	0.0 (2)
C2—N1—C1—N2	0.8 (2)	C5—N3—C9—C8	−0.3 (2)
C2—N1—C1—N4	−179.37 (13)	C7—C8—C9—N3	0.0 (2)
C10—N4—C1—N2	2.9 (2)	C1—N4—C10—C11	−3.0 (3)
C10—N4—C1—N2	2.9 (2)	C1—N4—C10—C15	176.64 (14)
C10—N4—C1—N1	−176.90 (14)	N4—C10—C11—C12	178.33 (13)
C1—N1—C2—C3	−0.8 (2)	C15—C10—C11—C12	−1.3 (2)
N1—C2—C3—C4	0.4 (2)	C10—C11—C12—C13	0.9 (2)
C1—N2—C4—N2	0 (100)	C10—C11—C12—N5	−178.38 (13)
N2—N2—C4—C3	0.0 (3)	O2—N5—C12—C13	−169.19 (15)
C1—N2—C4—C3	0.0 (2)	O1—N5—C12—C13	10.2 (2)
N2—N2—C4—C5	0.0 (3)	O2—N5—C12—C11	10.1 (2)
C1—N2—C4—C5	179.83 (12)	O1—N5—C12—C11	−170.51 (14)

C2—C3—C4—N2	0.0 (2)	C11—C12—C13—C14	-0.1 (2)
C2—C3—C4—N2	0.0 (2)	N5—C12—C13—C14	179.22 (13)
C2—C3—C4—C5	-179.82 (14)	C12—C13—C14—C15	-0.4 (2)
C9—N3—C5—C6	0.6 (2)	C13—C14—C15—C10	0.0 (2)
C9—N3—C5—C4	-179.53 (13)	C13—C14—C15—C16	-179.92 (14)
N2—C4—C5—N3	171.65 (13)	C11—C10—C15—C14	0.9 (2)
N2—C4—C5—N3	171.65 (13)	N4—C10—C15—C14	-178.77 (13)
C3—C4—C5—N3	-8.5 (2)	C11—C10—C15—C16	-179.22 (14)
N2—C4—C5—C6	-8.5 (2)	N4—C10—C15—C16	1.1 (2)

Hydrogen-bond geometry (Å, °) for (shelx)

<i>D</i> —H $\cdots$ <i>A</i>	<i>D</i> —H	H $\cdots$ <i>A</i>	<i>D</i> $\cdots$ <i>A</i>	<i>D</i> —H $\cdots$ <i>A</i>
C2—H2 $\cdots$ O1 ⁱ	0.95	2.59	3.3777 (19)	140
C9—H9 $\cdots$ O2 ⁱⁱ	0.95	2.51	3.1565 (19)	125
C11—H11 $\cdots$ N2	0.95	2.24	2.8723 (19)	124

Symmetry codes: (i) $x-1, y, z-1$; (ii) $x-1/2, -y+5/2, z-1/2$.

Interaction energies of pairs of molecules (kJ/mol) obtained from energy lattice calculations for the NPPA.

colour	N	Symmetry code	R	Electron density	E(El)	E(pol)	E(dis)	E(rep)	E(tot)
i	2	-x+1/2, y+1/2, -z+1/2	13.04	B3LYP/6-31G (d,p)	0.2	0.0	-1.1	0.0	-0.8
ii	1	-x,-y,-z	18.15	B3LYP/6-31G (d,p)	0.0	0.0	0.1	0.0	-0.1
iii	1	-x,-y,-z	15.22	B3LYP/6-31G (d,p)	0.0	0.0	-0.3	0.0	-0.2
iv	2	x+1/2, -y+1/2, z+1/2	10.51	B3LYP/6-31G (d,p)	-14.3	-4.1	-20	23.5	-21.0
v	2	x,y,z	11.89	B3LYP/6-31G (d,p)	-4.9	-1.4	-5.8	5.1	-8.2
vi	2	x,y,z	17.89	B3LYP/6-31G (d,p)	0.2	0.0	-0.1	0.0	0.1
vii	1	-x,-y,-z	10.54	B3LYP/6-31G (d,p)	-0.9	-0.2	-8.7	2.3	-7.3
viii	2	-x+1/2, y+1/2, -z+1/2	10.35	B3LYP/6-31G (d,p)	-6.8	-1.6	-20.1	15.5	-16.3
ix	1	-x,-y,-z	18.00	B3LYP/6-31G (d,p)	0.0	0.0	-0.1	0.0	-0.1
x	2	x+1/2, -y+1/2, z+1/2	10.97	B3LYP/6-31G (d,p)	-4.1	-2.4	-18.4	13.2	-14.0
xi	2	-x+1/2, y+1/2, -z+1/2	12.31	B3LYP/6-31G (d,p)	-2.4	-0.2	-1.2	0.0	-3.7
xii	1	-x,-y,-z	3.74	B3LYP/6-31G (d,p)	-7.8	-2.8	-99.2	54.4	-63.1
xiii	1	-x,-y,-z	4.39	B3LYP/6-31G (d,p)	-13.8	-2.3	-95.4	56.4	-64.6
xiv	2	-x+1/2, y+1/2, -z+1/2	9.96	B3LYP/6-31G (d,p)	-5.3	-0.9	-18.2	11.4	-15.1
xv	1	-x,-y,-z	9.48	B3LYP/6-31G (d,p)	-5.4	-2.3	-16.1	8.3	-16.4

xvi	1	-x,-y,-z	18.69	B3LYP/6-31G _{0.4} (d,p)	0.0	-0.1	0.0	0.3
xvii	1	-x,-y,-z	18.54	B3LYP/6-31G _{0.4} (d,p)	0.0	-0.1	0.0	0.3
xviii	1	-x,-y,-z	14.14	B3LYP/6-31G _{1.3} (d,p)	-0.1	-0.4	0.0	1.0

* Scaling factors used for the determination of the total energy E_{Tot}: E_{ee} = 1.057, E_{pol} = 0.740, E_{dis} = 0.871 and E_{rep} = 0.618, R is the distance between molecule centroids (Å), N is the number of pairs of molecules in the cluster with that particular interaction energy.

The most relevant hydrogen bonds formed by the protein residues of 2HYY with imatinib and NPPA.

Imatinib					NPPA		
Residue	Amino acid	H-A (Å)	D-A (Å)	Donor angle	H-A (Å)	D-A (Å)	Donor angle
286C	Glu	2.24	3.09	143.11			
315C	Thr	2.34	3.01	124.55	2.46	3.19	143.37
318C	Met	2.00	2.94	158.24			

IR Assignments and distribution of percent potential energy (%PED)

Assignments (PED%)	Exp (cm ⁻¹)	6311G++(d,p) (cm ⁻¹)	
		Without sc.	Sc.
ν NH (100)	3448	3631	3481
ν CH (99)	3145	3288	3152
ν CH (91)	3090	3115	2986
ν CH (100)	3063	3054	2928
ν CC (55)	1578	1655	1587
ν CC (14) + ν NC (12)	1553	1619	1553
ν _{as} ON (59)	1518	1575	1510
ν ON (16) + β HCC (22)	1474	1557	1492
β HCN(56)	1452	1514	1452
β HCC(42) + β HCH(78) + τ HCCC(15)	1430	1491	1429
ν NC(43)	1399	1459	1399
β HCN(42)	1380	1435	1376
ν _s ON (64)	1342	1367	1310
ν CC (12)	1308	1338	1283
ν NC (37) + β HCN(19)	1285	1322	1267
ν NC (30) + ν CC(10)	1263	1280	1227
ν NC (34) + β HCN(10)	1238	1264	1212
β CNC(51)	1115	1130	1083
ν CC (30) + β HCC (50)	1096	1119	1073
ν CC (33) + β HCH (11)	1077	1092	1047
ν CC (45)	1055	1065	1021
ν CC (21) + β CCC (45)	989	1012	970
ν NC (10) + τ HCCC (13) + β CCC(17)	963	981	941
τ HCCC(81)	888	924	886

τ HCNC(76)	847	880	843
δ ONO(43) + ν ON(11)	825	846	811
τ HCCC(79)	800	831	797
τ HCCC(70)	781	819	785
β CNC (52) + ν CC(11)	742	768	736
τ HCCC (10) + τ HCCN(69)	734	760	729
γ CCNC(70)	649	687	659
β CCC(68)	617	632	606

ν -Stretching; β -in-plane deformation; δ -scissors; γ -out-of-plane deformation; τ -torsion; s-symmetrical; as: asymmetrical; r -rocking; t -twisting.