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Expediting Drug Discovery: Fast and Accurate Prediction of Coupling Constants for Nitrogen Heterocycles

Petr Malek
College of Arts and Sciences, Boise State University

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Petr Malek, Owen McDougal PhD
Department of Chemistry and Biochemistry, Boise State University, Boise, Idaho, 83725

Abstract
Nitrogen containing heterocyclic drugs that regulate the JAK-STAT pathway have proven therapeutic usage for a variety of disorders from hematological cancers to rheumatoid arthritis. The synthesis of these drugs to selectively inhibit specific JAK-STAT has become an active area of research for pharmaceutical companies. Nitrogen heterocycles are commonly hydrogen deficient, presenting challenges for their characterization. The method of choice to determine the structure of these novel drugs is Heteronuclear Multiple Bond Correlation (HMBC) Nuclear Magnetic Resonance (NMR) spectroscopy. The limitation of this method is that it requires prior knowledge of the molecular orbital electron density of the molecule to be characterized. The NMR spectrometer must be tuned to the resonance frequencies of scalar coupled atom pairs multiple bonds removed from one another in order for their coupling to be observed. The problem to this trial & error approach to identify the correct coupling constants is that it requires significant & expensive spectrometer time. The experimentally determined HMBC NMR coupling constants shown here are being used to assess the complexity of computational ab initio electron density calculation necessary to a priori predict coupling constants for a range of nitrogen heterocycles. The degree of computational prediction sophistication to obtain accurate coupling constants will then be used to minimize the number of HMBC experiments for this class of compounds.

Background

Since the discovery of somatic Janus kinase (JAK) mutations in chronic myeloproliferative neoplasms, selective inhibition of JAKs have been shown to provide therapeutic effects for myeloproliferative disorders (Figure 1).[1-3]

Heteronuclear Multiple Bond Correlation (HMBC) Nuclear Magnetic Resonance (NMR) experiments allow identification and characterization of molecular structures for the Janus kinase family member drugs (Figure 2).[1,4] The long-range proton coupling constant obtained from analysis of an HMBC spectrum is used to identify the position of nitrogen atoms in a fused cyclic ring systems.[4,5] To account for the wide range of CCs in heterocyclic alkaloids, it is necessary to run numerous $^{1}H/^{15}N$ HMBC experiments, varying CC filter settings and decoupler offset frequency. ^{15}N has a relatively weak signal compared to ^{13}C, and $^{1}H/^{15}N$ HMBC experiments can often be in excess of ten times the duration of a satisfactory $^{1}H/^{13}C$ HSQC run.

To minimize the number of HMBC acquisitions needed to obtain good data for representative compounds, accurate estimates for $^{2}J_{HN}$, $^{3}J_{HN}$, etc. are determined *a priori*. In Spartan 10™ (Wavefunction Inc., Irvine, CA), geometry optimized structures of fused heterocyclic alkaloids are assembled,[6] representative of the classes of molecules commonly used as scaffolding for therapeutic drug synthesis.[3,4] Single point Hartree-Fock energy calculations are performed using increasingly large basis sets to determine the minimal theoretical prediction set size required for agreeable results between experimental and theoretical outcomes for the various classes of heterocyclic scaffolds.

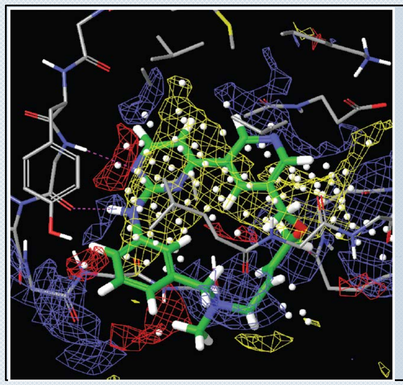

Figure 1: An illustration of JAK2 with highlighting hydrophobic regions (yellow), H-bond acceptor regions (blue), H-bond donor regions (red), and favorable ligand locations (white dots).

Results

Coupling between hydrogen and nitrogen has been characterized from several HMBC acquisitions. Figure 2 shows the structure of 6-bromoimidazo[1,2-a]pyrimidine. Each atom is labeled for correlation to the peak it represents in Figure 3. The labeling also allows the interpretation of which atoms within the structure are resonating (coupling) with one another (Table 1).

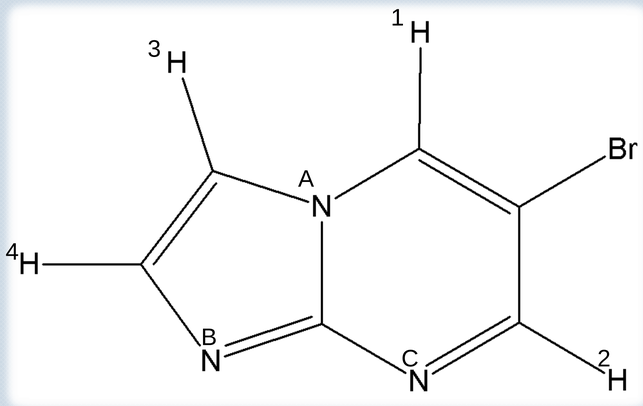

Figure 2: 6-bromoimidazo[1,2-a]pyrimidine.

Table 1: Correlation peaks & observed coupling constants.

6-bromoimidazo[1,2-a]pyrimidine	
Peak (^{1}H / ^{15}N)	$^{(2-3)}J$ (Hz)
4/A	3.80 ± 0.02
4/B	10.60 ± 0.02
3/A	4.29 ± 0.01
2/C	2.56 ± 0.06
1/A	1.36 ± 0.03

Discussion

Experimental values of $^{2}J_{HN}$, $^{3}J_{HN}$, etc. of the scaffold sample molecules were obtained (Figure 3) and these data are presented in Table 1. Also indicated is the number of bonds between atoms resulting in each correlation peak. The resulting *J* values indicate necessary coupling constant filter settings to maximize resonance, revealing multiple bond correlations.

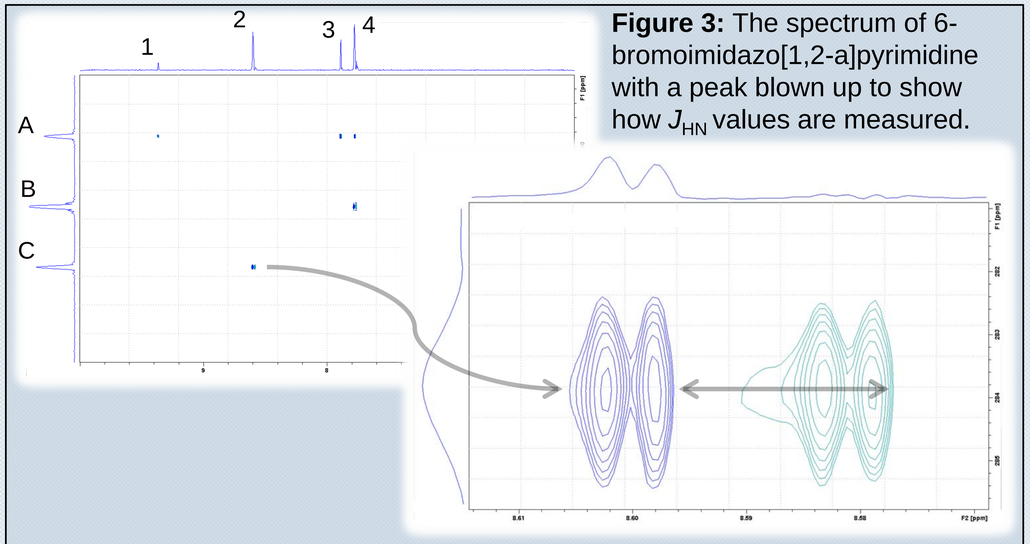

Figure 3: The spectrum of 6-bromoimidazo[1,2-a]pyrimidine with a peak blown up to show how J_{HN} values are measured.

Future Work

It is hypothesized that the addition of allowing manipulation of the raw density matrix data of molecular orbitals in Spartan 10[6,7] will permit ab initio calculations, minimizing bias from predetermined basis sets in previous versions of Spartan, to permit the establishment of empirical criteria for setting $^{1}H/^{15}N$ HMBC coupling filters *a priori*, minimizing the number of NMR experiments required for structure elucidation.

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References

1. Hanan, E. J.; van Abbema, A.; Barrett, K. Blair, W. S.; Blaney, J.; Chang, C.; Eigenbrot, C.; Flynn, S.; Gibbons, P.; Hurley, C. A.; Kenny, J. R.; Kulagowski, J.; Lee, L.; Magnuson, S. R.; Morris, C.; Murray, J.; Pastor, R. M.; Rawson, T.; Siu, M.; Ultsch, M.; Zhou, A.; Sampath, D.; Lyssikatos, J. P. Discovery of Potent and Selective Pyrazolopyrimidine Janus Kinase 2 Inhibitors. *J. Med. Chem.* **2012,** *55,* 10090-10107.
2. Baxter, J.; Scott, L. M.; Campbell, P. J.; East, C.; Fourouclas, N.; Swanton, S.; Vassilou, G. S.; Bench, A. J.; Boyd, E. M.; Curtin, N.; Scott, M. A.; Erber, W. N.; Green, A. R. Acquired Mutation of the Tyrosine Kinase JAK2 in human myeloproliferative disorders. *Lancet.* **2005,** *365,* 1054-1061
3. William, A. D.; Lee, A. C.-H.; Poulsen, A.; Goh, K. C.; Madan, B.; Hart, S.; Tan, E.; Wang, H.; Nagaraj, H.; Chen, D.; Ping. C.; Sun, E. T.; Jayaraman, R.; Pasha, M. K.; Ethirajulu, K.; Wood, J. M. Discovery of the Macrocycle (9E)-15-(2-(Pyrrolidin-1-yl)ethoxy)-7,12,25-trioxa-19,21,24-triaza-tetracyclo[18.3.1.1(2,5).1(14,18)]hexacosa-1(24),2,-4,9,14(26),15,17,20,22-nonaene (SB1578), a Potent Inhibitor of Janus Kinase 2/Fms-LikeTyrosine Kinase-3 (JAK2/FLT3) for the Treatment of Rheumatoid Arthritis. *J. Chem. Med.* **2012,** *55,* 2623-2640.
4. Chandrashekhar, N.; Thomas, B.; Gayathri, V.; Ramanathanb, K. V.; Nanje Gowdaa. N.M. Synthesis and NMR Spectral Assignments of Novel Nitrogen and Sulfur Heterocyclic Compounds. *Magn. Reson. Chem.* **2008,** *46,* 769-774.
5. Al-Soud, Y. A.; Al-Masoudi, N. A. Structural Assignments of 1-(β-D-Glucopyranosyl)-1,2,3-triazoles by ^{1}H- and ^{13}C-NMR Study. Dekker, **2003,** 36, 461-475.
6. Hehre, W. J. A Guide to Molecular Mechanics and Quantum Chemical Calculations. http://www.wavefun.com/support/AGuidetoMM.pdf (accessed Sep. 25, 2013).