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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

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/$^{13}$N HSQC run.

To minimize the number of HMBC acquisitions needed to obtain good data for representative compounds, accurate estimates for $J_{HN}$, $J_{AB}$, etc. are determined a priori. In Spartan 10TM (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.

Future Work
It is hypothesized that the addition of allowing manipulation of the raw density matrix data of molecular orbitals in Spartan 10.7 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
5. Al-Soud, Y. A.; Al-Masoudi, N. A. Structural Assignments of 1,3-DiGlycosyluracil 1,3-DiNucleosides by 1H- and 13C-NMR Study. Oikakis 2003, 36, 431-435.

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).

Figure 2: The spectrum of 6-bromimidazo[1,2-α]pyrimidine.

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

Table 1: Correlation peaks & observed coupling constants.

<table>
<thead>
<tr>
<th>6-bromimidazo[1,2-α]pyrimidine</th>
<th>$^1$H ($^15$N)</th>
<th>$^2$J ($^1$H) (Hz)</th>
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<tbody>
<tr>
<td>4/A</td>
<td>3.80 ± 0.02</td>
<td>3.00 ± 0.05</td>
</tr>
<tr>
<td>4/B</td>
<td>10.60 ± 0.02</td>
<td>3.00 ± 0.05</td>
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<tr>
<td>3/A</td>
<td>4.29 ± 0.01</td>
<td>3.00 ± 0.05</td>
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<tr>
<td>2/C</td>
<td>2.56 ± 0.06</td>
<td>3.00 ± 0.05</td>
</tr>
<tr>
<td>1/A</td>
<td>1.36 ± 0.03</td>
<td>3.00 ± 0.05</td>
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