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A ^{13}C NMR approach to categorizing potential limitations of α,β-unsaturated carbonyl systems in drug-like molecules

Kevin P. Cusack,* Lee D. Arnold,† Claude E. Barberis, Haipeng Chen, Anna M. Ericsson, Georgeen S. Gaza-Bulseco, Thomas D. Gordon, Christine M. Grinnell, Andreas Harsch, Maria Pellegrini‡ and Edit Tarcsa

Abbott Bioresearch Center, 381 Plantation Street, Worcester, MA 01605, USA

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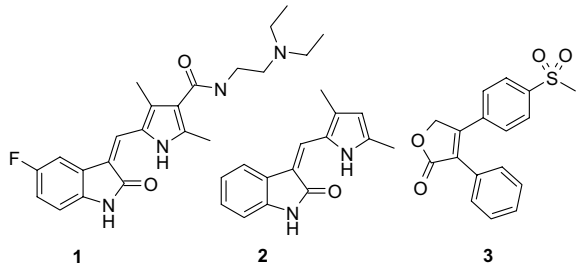
Abstract—Compounds that contain an α,β-unsaturated carbonyl moiety are often flagged as potential Michael acceptors. All α,β-unsaturated carbonyl moieties are not equivalent, however, and we sought to better understand this system and its potential implications in drug-like molecules. Measurement of the ^{13}C NMR shift of the β-carbon and correlation to in vitro results allowed compounds in our collection to be categorized as potential Michael acceptors, potential substrates for NADPH, or as photoisomerizable.

The use of an aldol-type condensation reaction to rapidly generate libraries of small molecules can be very attractive, as many aldehydes and activated methylene compounds are readily available.[1] However, the resultant products often contain an α,β-unsaturated carbonyl moiety. Though these compounds do not directly violate the 'rule of five',[2] they are commonly flagged as less than optimal leads due to their potential to act as Michael acceptors.[3] It should be noted, however, that compounds containing an α,β-unsaturated carbonyl moiety have made it as far as the clinic including most recently the PDGFR inhibitor SU 11248 **1** and the related VEGFR inhibitor SU 5416 **2** (Fig. 1).[4] Though these compounds are only in clinical trials, **2** is noted to have been administered to two patients for greater than 18 months with no evidence of cumulative toxicity.[5a] Other α,β-unsaturated carbonyl containing molecules have advanced to become successful drugs such as Vioxx **3**.[5b] Still others, such as drugs that have been designed to act as irreversible inhibitors,[6] forming a covalent bond between a nucleophilic residue in the active site and the β-carbon of the inhibitor, will not be discussed here.

In order to better understand the potential limitations of our compounds that contained an α,β-unsaturated carbonyl moiety, we began looking for a predictive tool that could be used to rank scaffolds relative to one another. Measurement of the ^{13}C NMR signal of the β-carbon was explored as a way to estimate the electrophilicity[7] and any potential limitations associated with a reactive center. We found that observation of the ^{13}C NMR signal of the β-carbon placed each scaffold into a distinct group that showed little overlap with other groups. Each scaffold was in turn found to have distinct physical properties that distinguished it from



1 2 3

Figure 1. Known α,β-unsaturated inhibitors.

Keywords: Michael acceptor; Electrophilicity; α,β-unsaturated; NMR; Physical properties.
* Corresponding author. Tel.: +1 508 688 8006; fax: +1 508 688 8100; e-mail: kevin.cusack@abbott.com
† Present address: OSI Pharmaceuticals, 1 Bioscience Pk. Dr., Farmingdale, NY 11735, USA.
‡ Present address: Biogen Inc., 14 Cambridge Center, Cambridge, MA 02142, USA.

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other scaffolds studied. In the pyrrolo-pyrazolinone group **B** as exemplified by **6**, subtle variations within this group even tracked with the ^{13}C NMR shift of the β-carbon. Following experimental confirmation, it became apparent that compounds (or in this case, scaffolds) could be classified as potential Michael acceptors, potential substrates for NADPH, or as potential photoisomerizable compounds by simply observing the ^{13}C NMR shift of the β-carbon. This measurable physical property allowed us to quickly classify potential limitations of each scaffold.

The scaffolds shown in Figure 2 represent the three classes studied. In all cases, an α,β-unsaturated carbonyl moiety is generated during synthesis (Scheme 1).

All compounds were evaluated against our panel of enzymes in vitro and an inconsistent SAR pattern began to emerge. In particular, it was discovered that the pyrazolinones **A** (Fig. 2) were much less potent than analogous pyrazolinones **B** (Fig. 2). Further analysis into the cause of this apparent shift in potency led us to explore the possibility that these compounds could be acting as Michael acceptors of nucleophiles in our in vitro assay protocol[8] as loss of the enone system was known to be detrimental to activity. The assay conditions employed required the addition of dithiothreitol (DTT) in order to reduce disulfide bond formation. By simply eliminating DTT from our assay, the expected SAR pattern emerged and the anticipated increased relative potency was observed (Table 1). This was the first in vitro confirmation of the Michael accepting potential of this class of compounds.

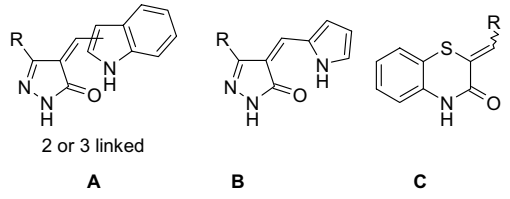
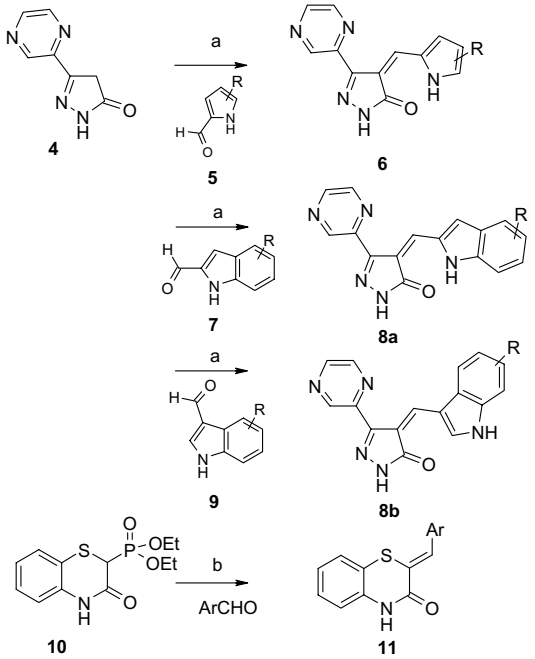


Figure 2. Representative scaffolds.



Scheme 1. (a) Piperidine (catalytic), EtOH, 60–80 °C; (b) NaOMe, MeOH, 12 h.

Even more intriguing to us was the same relationship between activity and DTT did not emerge for compounds of type **B** or **C** (Table 2). As is evident from the data in Table 2, when compounds do not show sensitivity to DTT, the observed potency drops off. In this case enzyme efficiency is compromised presumably due to disulfide bond formation, which is reduced by DTT.

The β-carbon in each scaffold was identified by sequential NMR analysis as described in the literature and in the representative example here.[9] In order to unambiguously establish which singlet was the vinyl proton (e) a heteronuclear correlation experiment (HMBC) was run. A through-bond correlation of H_e to the ^{13}C assigned carbonyl carbon was observed (Fig. 3). Further evidence came from a 2D ROESY experiment where cross-peaks of both H_g to H_e (strong) and H_f to H_a (weak) were noted (consistent with distances from our *Z*-conformation model). H_g was assigned via the chemical shift and ^1H–^1H coupling constants, as well as TOCSY and ROESY correlation to a signal consistent with H_p. H_a was also correlated via TOCSY and ROESY experiments to protons H_b–H_d. This allowed us to easily distinguish between H_f and H_e and thus assign the β-carbon in our compounds.

Table 1. Effect of DTT on measured KDR potency for class **A** compounds (1 mM ATP)

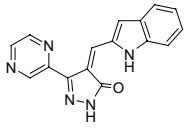
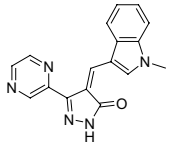
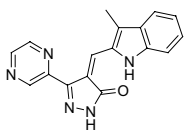
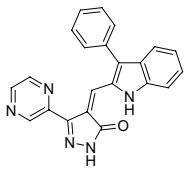
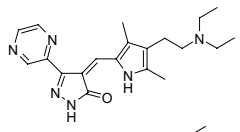
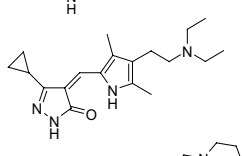
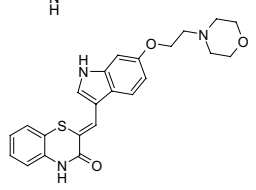
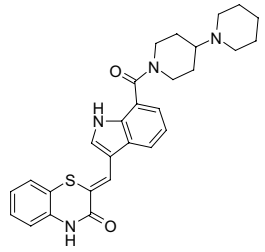
Entry	Structure	IC_{50} (μM) (+DTT)	IC_{50} (μM) (−DTT)
A1		40.01	0.41
A2		0.23	0.10
A3		0.14	0.06
A4		0.47	0.02

Table 2. Effect of DTT on measured KDR potency for class **B** and class **C** compounds (1 mM ATP)

Entry	Structure	IC_{50} (μM) (+DTT)	IC_{50} (μM) (−DTT)
B1		0.08	0.21
B2		0.84	2.50
C1		0.16	0.29
C2		0.17	0.72

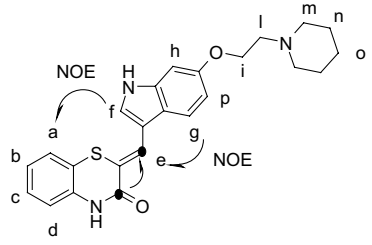


Figure 3. Typical NMR correlation.

Upon examination of the ^{13}C NMR shift of the β-carbon we noted a downfield shift to 137–139 ppm in compounds of type **A** relative to compounds of type **B** or **C** (Table 3). Further examination of a broader data set revealed that the compounds of type **B** were grouped together at 128–135 ppm and downfield of compounds of type **C** at 118–122 ppm giving us three distinct ^{13}C NMR ranges that correlate well with the scaffold studied. Closer examination of each scaffold revealed that more subtle trends could exist within individual scaffolds.

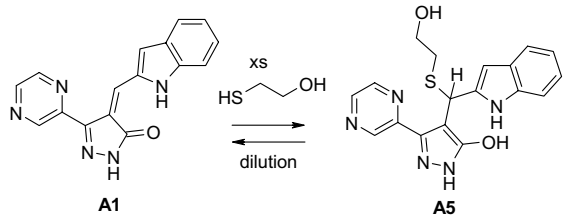
Compounds identified as potential Michael acceptors (Type **A**) showed consistent shifts in our in vitro assay system depending on whether DTT was present. In order to chemically confirm a Michael addition had occurred, the addition of β-mercaptoethanol (BME) was studied on an NMR time scale. Concentration dependent equilibrium between unbound **A1** and bound **A5** forms was observed. Elevated concentrations of BME were sufficient for observation of adduct formation with the key proton signal appearing at 6.4 ppm (alpha to sulfur).[10a] Examination of stoichiometric amounts of BME or dilution of the concentrated samples showed only starting material by ^{1}H NMR and MS. This indicated that the addition was both reversible (Scheme 2) and concentration dependent.

Interesting was the fact that both 2- and 3-substituted indoles **A** (Fig. 2) behaved similarly. No appreciable photoisomerization was seen with compounds of this structural class regardless of whether they were 2- or 3-linked at indole (i.e., photostability via an internal H-bond was not required).

Unlike compounds of Type **A**, compounds of Type **B** were not susceptible to DTT shifts. However, these compounds did show higher (though limited) rates of in vitro metabolism in S9 and microsomal preparations that contained high concentrations (5 mM) of NADPH.[10b] Control reactions that were carried out in buffer with NADPH (2 mM, Fig. 4) in the absence of

Table 3. Classification of scaffolds

Class	Michael acceptor	Reduction potential	Photoisomerize	C-13 β-carbon (ppm)
A	+++	–	–	137–139
B	–	+++	–	128–135
C	–	+	+++	118–122



Scheme 2. Thiol addition to class **A** compounds.

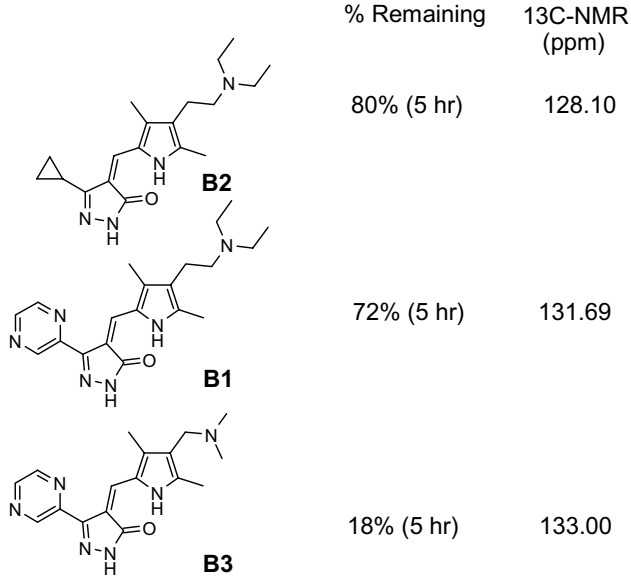


Figure 4. Percent parent remaining after incubation with NADPH.

microsomes also showed a time and NADPH dependent loss of color. This led us to believe the reduction of the double bond was a potential nonenzymatic reaction product. The ability to quantify the reduction products was made difficult due to the capacity of these compounds to rapidly re-oxidize in air.[10d]

This series (Type **B**) was of great interest since the ^{13}C NMR chemical shift of the β-carbon sits between potential Michael acceptors (Type **A**) and known photoisomerizable compounds (Type **C**). As such, we were interested in the trend within this class. The data demonstrates what appears to be good correlation between the chemical shift of the β-carbon and the degree of in vitro reactivity with NADPH.[10c] Indeed, we found reduced reactivity with NADPH by simply altering either the pyrazine ring (to cyclopropyl) or the chain length to the basic amine (Fig. 4). This strategy resulted in the identification of analogs with reduced metabolic potential and no evidence of acting as Michael acceptors in NMR studies. Photostability in this case is achieved due to internal H-bond formation.[9] In an effort to assess the toxicity potential of compounds of Type **B**, we evaluated their ability to inhibit cellular proliferation. These compounds were shown not to inhibit cell growth in vitro suggesting their ability to act as substrates for NADPH at physiological NADPH concentrations is limited, and did not result in mechanistic toxicity in our cellular assay.

The third class of compounds represented by the benzothiazinones **C** did not appear to be as susceptible to reduction by NADPH or to be a capable Michael acceptor, but did photoisomerize to varying degrees. The equilibrium ratio varied from 0% to 10% of the *E*-isomer.[11a] This is the only scaffold where the line was a bit vague between the neighboring pyrrolopyrazolinone scaffold **B**. That is, some benzothiazinones were photoisomerizable and susceptible to reduction. Once again, however, compounds of type **C** did not appear to be mechanistically toxic in our cellular proliferation assay.

Not every result was predictable, however. An unanticipated result was observed within the benzothiazinone scaffold **C**. In investigating the photostability of various ring systems attached to the core benzothiazinone, we noted that an imidazole ring with an unsubstituted N-1 was completely photostable presumably due to H-bonding to S or O. In support of a H-bond, the N-1 position of imidazole **C3** was methylated and lost all photostability. In a second approach, we were also able to disrupt the photostability by tethering a proton source such as a propionic acid to the 2-position of the imidazole. It is hypothesized that either zwitterion formation or an internal H-bond to the carboxylic acid is established essentially disrupting the H-bond to the core. The β-carbon for imidazole analogs such as **C3** is typically ~120 ppm (Fig. 5).

In summary, we have shown a correlation between observed chemical shift of the β-carbon of α,β-unsaturated carbonyl systems and physical behavior of these structurally unique scaffolds. Though this behavior is difficult

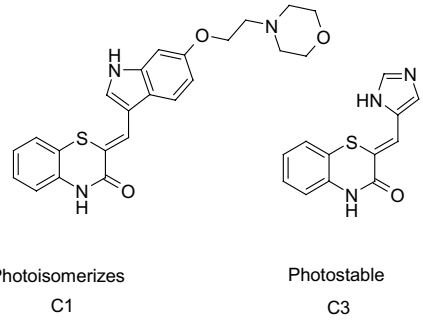


Figure 5. Compounds of class **C**.

to directly correlate to any observable toxicity, the fact that we are able to rationalize compound behavior is beneficial to assessing the value of each scaffold.[12] This assessment led us to identify the pyrrole pyrazolinones **B** as the series with the best overall properties, consistent with the ^{13}C NMR shifts of known indolinones **1** and **2** that have been chronically dosed in humans. It is noteworthy that we were able to identify potent compounds that were photostable, had reduced potential to reduction and showed no ability to act as Michael acceptors using this approach (compounds **B1** and **B2** for example).

Acknowledgements

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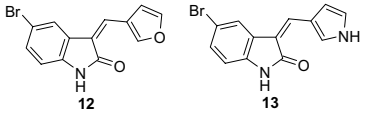
References and notes

1. For examples (a) Mead, D.; Loh, R.; Asato, A. E.; Liu, R. S. H. *Tetrahedron Lett.* **1985**, *26*(24), 2873; (b) Willert, M.; Benito, J. M.; Meldal, M. *J. Comb. Chem.* **2003**, *5*, 91; (c) Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; McMahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2588; (d) Adams, C.; Aldous, D. J.; Amendola, S.; Bamborough, P.; Bright, C.; Crowe, S.; Eastwood, P.; Fenton, G.; Foster, M.; Harrison, T. K. P.; King, S.; Lai, J.; Lawrence, C.; Letallec, J.-P.; McCarthy, C.; Moorcroft, N.; Page, K.; Rao, S.; Redford, J.; Sadiq, S.; Smith, K.; Souness, J. E.; Thurairatnam, S.; Vine, M.; Wyman, B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3105; (e) Paterson, I.; Temal-Laieb, T. *Org. Lett.* **2002**, *4*, 2473.
2. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
3. Hann, M.; Hudson, B.; Lewell, X.; Lifely, R.; Miller, L.; Ramsden, N. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 897; Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. *Chem. Res. Toxicol.* **2000**, *13*(3), 135.
4. Fabbro, D.; Garcia-Echeverria, C. *Curr. Opin. Drug Discovery Dev.* **2002**, *5*(5), 701.
5. (a) Rehnowe, P. A. *Curr. Opin. Drug Discovery Dev.* **2002**, *5*, 214; (b) The ^{13}C NMR shift of the β-carbon is reported to be 155.9 ppm and labeling studies indicated ring oxidation as a primary metabolic pathway. See Baillie, T. A.; Halpin, R. A.; Matuszewski, B. K.; Geer, L. A.; Chavez-Eng, C. M.; Dean, D.; Braun, M.; Doss, G.; Jones, A.; Marks, T.; Melillo, D.; Vyas, K. P. *Drug Metabol. Dispos.: Biol. Fate Chem.* **2001**, *29*(12), 1614.

6. Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, H.; Reyner, E. L.; Lipka, E.; Hollis Showalter, H. D.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. *J. Med. Chem.* **2000**, *43*, 1380.
7. ^{13}C NMR has previously been used to estimate the electrophilicity of carbon: Spencer, S. R.; Xue, L. A.; Klenz, E. M.; Talalay, P. *Biochem. J.* **1991**, *273*, 711; (b) Ding, J. *Daxue Huaxue* **1994**, *9*, 51.
8. All compounds were synthesized and evaluated as described: Rafferty, P.; Calderwood, D.; Arnold, L. D.; Gonzalez Pascual, B.; Ortego Matinez, J. L.; Perez de Vega, M. J.; Fernandez, I. F. PCT Int. Appl. WO0075139 A2, 2000; Moset, M. M.; Berlanga, J. M. C.; Fernandez, I. F.; Calderwood, D. J.; Rafferty, P.; Arnold, L. D. PCT Int. Appl. WO0109121A2, 2001.
9. (a) Similar to evidence noted in Ref. 1c above; (b) All NMR data were acquired on a Bruker Avance 400 MHz spectrometer at a temperature of 25 °C, and referenced to internal TMS. Samples were dissolved in DMSO-d_6 (CIL) at final concentrations of 1–10 mM; (c) HMBC Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093; (d) TOCSY Bax, A.; Davis, G. G. *J. Magn. Reson.* **1985**, *65*, 355; (e) ROESY Bax, A.; Davis, G. G. *J. Magn. Reson.* **1985**, *63*, 207.
10. (a) Adduct formation resulted in a pattern consistent with enolization of the pyrazolinone core to the hydroxy form analogous to core prior to condensation with aldehydes; (b) Microsomal incubations are run with 10 μM compound (0.1% DMSO final concentration in assay), incubated in the assay buffer (0.1 M Tris HCl, pH 7.5, 5 mM $MgCl_2$) with 5 mM NADPH (Sigma) and 1 mg/mL mouse microsomes or S9 (In Vitro Technologies) at 37 °C for 1 h; (c) To assess the reactivity of selected compounds with NADPH, control reactions were carried out with 50 μM compound, 2 mM NADPH in 50% ethanol, 50% assay buffer at ambient temperature for 5 h; (d) Loss of parent observed, reduced form difficult to quantify.
11. (a) Ratio of *E* and *Z* isomers was estimated via analytical LC and ^1H NMR ratios; (b) While most benzothiazinones **C** photoisomerized <10%, a related indolinone scaffold (**12**, **13** for example) could display broader equilibrium ratios (10–50% of the minor isomer). The observed chemical shift for the β-carbon is typically 127–134 ppm (similar to **B** and outside the furthest downfield value observed for benzothiazinones of type **C**). As comparison, a typical benzothiazinone (Table 2, entry 4) isomerizes ~10% in 24 h (1 mg/mL DMSO, room light, 120 ppm typically observed) while indolinone **13** isomerizes 25% in less than 1 h under similar conditions (134 ppm observed for **13**). In a 2-linked pyrrole analog of example **13** we noted complete photostability as observed in the literature[1c] due to the ability of the pyrrole-NH to form an internal H-bond (127 ppm observed for β-carbon).



12. C NMR data from unrelated scaffolds is consistent with these findings. Though not confirmed to be Michael acceptors, the level of substitution on the olefin and the carbonyl can shift the value of the β-carbon slightly outside the ranges observed in this paper. However, known Michael acceptors such as acrylonitrile fall within the observed range. For example, see Loots, M. J.; Weingarten, L. R.; Levin, R. H. *J. Am. Chem. Soc.* **1976**, *98*(15), 4571; Monge, S.; Selambarom, J.; Roque, J. R.; Pavia, A. A. *Tetrahedron* **2001**, *57*, 9979.