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Brief Article

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A Simple Litmus Test for Aldehyde Oxidase Metabolism of Heteroarenes

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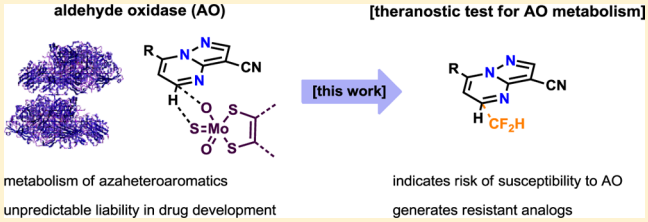
Supporting Information

ABSTRACT: The bioavailability of aromatic azaheterocyclic drugs can be affected by the activity of aldehyde oxidase (AO). Susceptibility to AO metabolism is difficult to predict computationally and can be complicated in vivo by differences between species. Here we report the use of bis-(((difluoromethyl)sulfinyl)oxy)zinc (DFMS) as a source of CF_2H radical for a rapid and inexpensive chemical "litmus test" for the early identification of heteroaromatic drug candidates that have a high probability of metabolism by AO.



INTRODUCTION

Over 93% of small molecule drug candidates fail in phase I–III clinical trials, and around 30% of the time this is due to unpredictable toxicity or clinical safety reasons.[1] Thus, methods for the rapid prediction of potential liabilities are in great demand among medicinal chemists. Along these lines, metabolism of heteroarenes by aldehyde oxidase (AO) is increasingly being recognized as an important factor.[2] There are well-documented cases of unanticipated susceptibility to this enzyme, leading to challenges in reproducing efficacious exposure between animal models and humans, and in some cases this has led to the termination of drug discovery programs. For example, the development of both Carbazeran[3] and SGX-523[4] were both abandoned at a late stage after testing in animals failed to anticipate the outcome in humans. In contrast to metabolism by cytochrome P450 (CYP),[5] there is limited precedent for methods to predict how susceptible a substrate may be to AO metabolism and it has also proven difficult to correlate substrate stability to AO in silico.[6] In this article, the invention of a simple chemical litmus test for the rapid (ca. 2 h) evaluation of the susceptibility of a heteroarene-containing drug candidate toward AO metabolism (Figure 1) is reported. This technologically straightforward chemical method acts as a surrogate for a more costly and labor-intensive biotransformation test and has already been field-tested at Pfizer.

Aldehyde oxidase is capable of metabolizing a number of different functionalities such as aldehydes and iminium ions but of greatest relevance to drug development is the oxidation of aromatic azaheterocycles such as pyridines, diazines, benzimidazoles, purines, and a wide variety of other fused heteroaromatic systems.[7] These electron-deficient heterocycles are common fragments in drugs because they are relatively resistant to metabolism by CYP but are often substrates for AO, where they

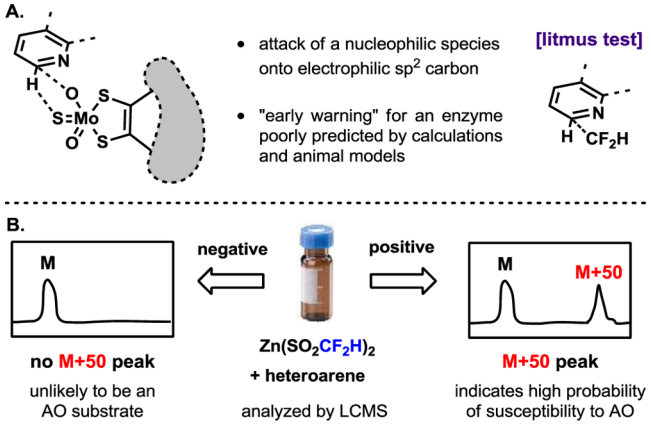


Figure 1. Concept and development of a simple chemical "litmus test" to predict susceptibility to AO metabolism. (A) Nucleophilic radical addition to heteroarenes as a model for AO activity. (B) "Litmus test" to alert of a high risk of AO metabolism on heteroaromatics.

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are typically oxidized adjacent to nitrogen to give pyridone-like species.[2a]

Oxidation by AO is thought to proceed by nucleophilic attack of a high-valent molybdenum−oxo species on aromatic carbon atoms adjacent to nitrogen (Figure 1A),[8] with the likelihood of AO metabolism being related to the susceptibility of the heterocycle to nucleophilic attack at this position. This implies that susceptibility to AO should be related to the electronic properties of the heteroarene at these sites, but as yet, attempts to correlate reactivity trends with AO with calculations of energies or electronic properties have not proven successful.[9] At present, prediction of whether or not a structural feature introduces a potential liability to AO is limited to visual identification of specific structural features such as heteroarenes.[2a] Although valuable for simple systems, this approach has major limitations when considering fused azaheterocyclic systems and also when assessing the likely impact of introducing heterocyclic substituents on AO metabolism.

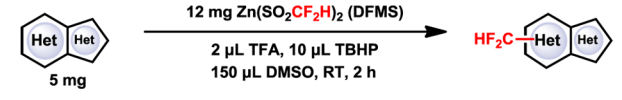
Recent findings on the direct C−H functionalization of pharmaceutically active heteroarenes using radicals derived from alkylsulfinate salts revealed strikingly predictable reactivity patterns.[10] A survey of known AO substrates revealed a close correlation between positions labile to AO oxidation and those that would be predicted as functionalizable by nucleophilic radicals. This is perhaps not unexpected, as the process of nucleophilic attack on an aromatic carbon atom, followed by cleavage of the C−H bond to regain aromaticity, is similar to the proposed mechanism for AO. As with AO metabolism, it has also proven difficult to correlate calculated electronic values of the heterocyclic substrates with observed reactivity trends with alkylsulfinate salts.[10] Our hypothesis was that the empirical reactivity of an azaheteroarene toward a nucleophilic radical species might closely approximate the susceptibility to AO-based metabolism. The aim of this work was to develop a simple chemical test to alert medicinal chemists of a high probability that a heteroaromatic structure may be prone to AO metabolism so that early biological testing may be prioritized.

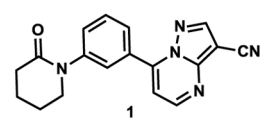
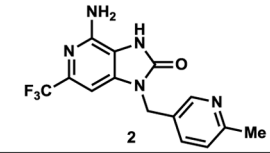
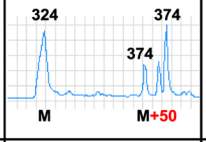
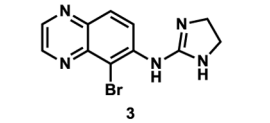
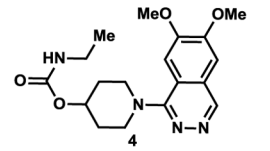
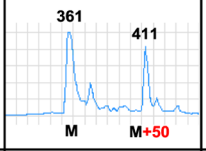
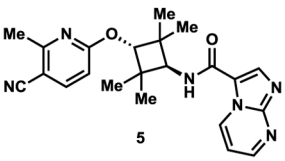
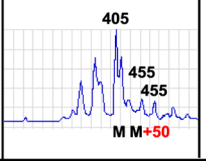
RESULTS AND DISCUSSION

The following requirements were deemed necessary for a practical and robust litmus test (Figure 1B): (i) the use of readily available reagents that required no special handling in terms of air or moisture, (ii) operational simplicity and straightforward analysis, (iii) reaction conditions that could tolerate the wide range of functional groups found in drug candidates, and (iv) robust and forgiving to weighing errors on small scale. Bis(((difluoromethyl)sulfinyl)oxy)zinc (DFMS)[11] was selected as the radical source as it has good reactivity at ambient temperature, the CF_2H radical is nucleophilic in character, and the difluoromethylated products are almost always more lipophilic than the parent compound, with LCMS analysis giving clearly differentiated peaks. A further advantage of DFMS is that it installs a small group that is relatively resistant to metabolism at the site previously prone to oxidation. Thus, if a drug candidate is prone to AO metabolism, it is possible to scale up and isolate these metabolically blocked compounds and retest them for improved qualities.

A set of five known AO substrates (**1**−**5**) identified from the literature were subjected to reaction with DFMS, and the crude reaction mixtures were examined by LCMS for the characteristic M+50 peak associated with the addition of a $-CF_2H$ group (Table 1). After extensive screening of reaction conditions, the protocol in Table 1 was developed as a modification to our

Table 1. Optimized "Litmus Test" Conditions for Five Known AO Substrates[a]



	molecule	LCMS TIC chromatogram	AO?	DFMS?
1			Y	Y
2			Y	Y
3			Y	Y
4			Y	Y
5			Y	Y

[a]Conditions were chosen based on simplicity and robustness of experimental protocol, and to limit the occurrence of side reactions. Analyzed by LCMS; TIC (total ion current) chromatogram shown, peaks of the protonated heteroarene (M) and difluoromethylated analogues (M+50) indicated.

standard conditions. DMSO was employed as the solvent to enhance solubility of drug-like compounds, and TFA was added to promote the "innate" reactivity pattern.[10] The reaction was carried out at room temperature to enhance tolerance of elaborate functionality and avoid multiple substitution, which could make the LCMS trace difficult to interpret. A binary qualitative answer (reactive or not reactive) was the desired readout of this litmus test. As such, all reagents could be employed in modest excess, and it was possible to carry out the test with about 5 mg of substrate (MW = 300−600), or a spatula tip, of the molecule of interest.

An expanded set of compounds, including both those known to be AO substrates and those that had been designed not to be susceptible, were subjected to the newly designed litmus test (Table 2). Known AO substrates **6** (SGX-523)[4] and zoniporide analogue **7**[12] gave clear M+50 peaks, indicating a positive test result. To verify the accuracy of the litmus test, a series of analogues of **5** designed when probing the effect of structural variation on AO metabolism[13] (**8**−**11**) were examined. While AO substrates **5** and **10** showed clear M+50 peaks on LCMS, indicating a positive result, **8**, **9**, and **11**, which had been designed to be AO-resistant, showed no or negligible levels (<10% of M peak) of the M+50 product. The predictive capability of our test

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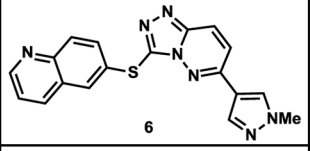
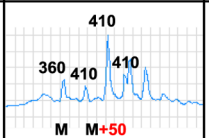
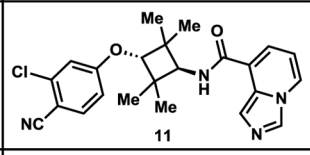
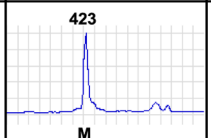
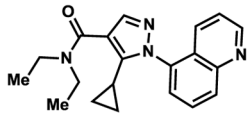
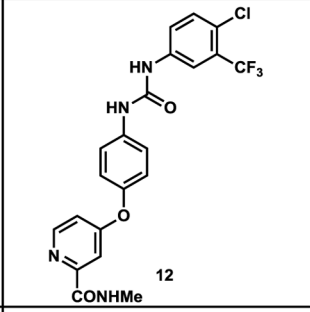
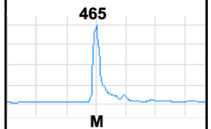
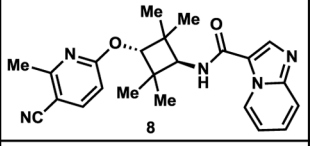
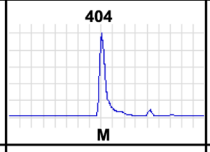
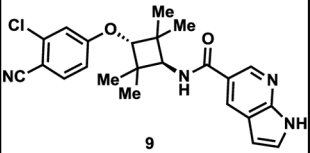
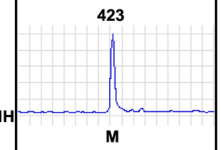
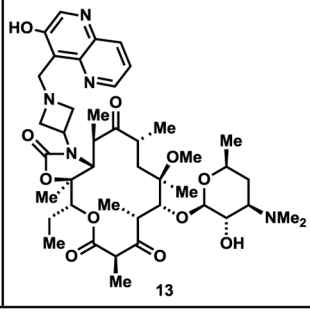
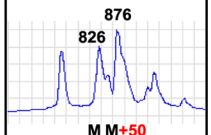
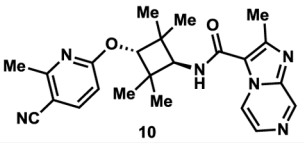
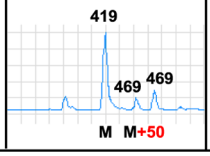
Table 2. "Litmus Test" DFMS Test Gave Results That Closely Matched Those of AO[a]

	molecule	LCMS TIC chromatogram	AO?	DFMS?		molecule	LCMS TIC chromatogram	AO?	DFMS?
6			Y	Y	11			N	N
7			Y	Y	12			N	N
8			N	N					
9			N	N	13			N	Y
10			Y	Y					

[a]Standard conditions: 5 mg of substrate, 12 mg of DFMS, 150 μL of DMSO, 2 μL of trifluoroacetic acid (TFA), and 10 μL of TBHP (70% aq solution), stirred for 2 h at RT. Samples were diluted with MeOH and analyzed by LCMS (TIC chromatogram shown). Peaks of the protonated heteroarene (M) and the difluoromethylated analogues (M+50) are indicated if present. In general, "positive" results gave clear evidence of reactivity with DFMS, and "negative" results gave no or negligible (<10% of the M peak) evidence of the difluoromethylated products (see Supporting Information for further details, full LCMS chromatograms, and additional examples of compounds tested).

was further explored on sorafenib (**12**), which we expected to be unreactive with DFMS[10] and had never before been tested for AO susceptibility. As predicted, **12** gave a negative litmus test result and subsequent metabolic testing confirmed resistance to AO oxidation.

AO susceptibility can be impacted by structural changes that affect enzyme binding, even if these changes are remote from the heteroarene.[12,14] A chemical reagent such as DFMS cannot mimic these aspects of the enzymatic reaction, thus "false positive" results may occur, as exemplified by ketolide antibiotic **13**, which was designed as an AO-resistant analogue in a series of ketolide antibiotics that varied only in their heteroarene fragment.[15] Although the "litmus test" indicated that the heteroarene fragment may be susceptible to AO oxidation, it is known that **13** is not an AO substrate,[16] possibly due to the precise shape of the heteroarene precluding AO binding. A further implication of the inability to mimic binding is that the DFMS litmus test is somewhat less discriminating than AO, giving rise to several isomers or multiple addition products, whereas AO is often selective for a single site.

Despite these limitations, the strong correlation between the litmus test results and susceptibility to AO oxidation is supportive of the use of DFMS as a rapid and simple "litmus test" to alert of the possibility of AO metabolism and decide whether to subject the compound or fragment to more detailed testing. We envision that this test may be especially valuable for assessing series of compounds of similar shape but with varying heteroarene fragments.

If a compound of interest proves to have unacceptable levels of AO metabolism, this can sometimes be mitigated using alternative isosteric heteroaromatic templates or by installing functionality to diminish AO activity at the metabolically susceptible position.[9,13] Installing a blocking group at the position oxidized by AO is the simplest approach, and an important advantage of the "litmus test" is the ease with which such derivatives can be obtained. Although electron-deficient heteroarenes can be difficult to elaborate, direct functionalizations mediated by nucleophilic radicals are both innately reactive toward these substrates and naturally selective for the desired position. DFMS allows for the convenient installation of CF_2H as a small blocking group that is unlikely to introduce further metabolic liabilities into the molecule. Repeating the "litmus test" at 0.125 or 0.25 mmol scale, with heating to 50 °C if required to enhance conversion, followed by separation by preparative HPLC, gave rapid access to **14**−**23** as the major products. These compounds, which typically have been selectively metabolically blocked at the position vulnerable to AO, were subjected to an AO activity assay and in all cases except **20** were no longer AO substrates. Although CF_2H may not be the final group that is installed in this position, this test provides a rapid verification that a blocking group in a specific position will produce the desired effect without necessitating laborious analogue synthesis.

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An interesting aspect of the electronic changes introduced by difluoromethylation is seen in **14** and **15** (Figure 2). Our

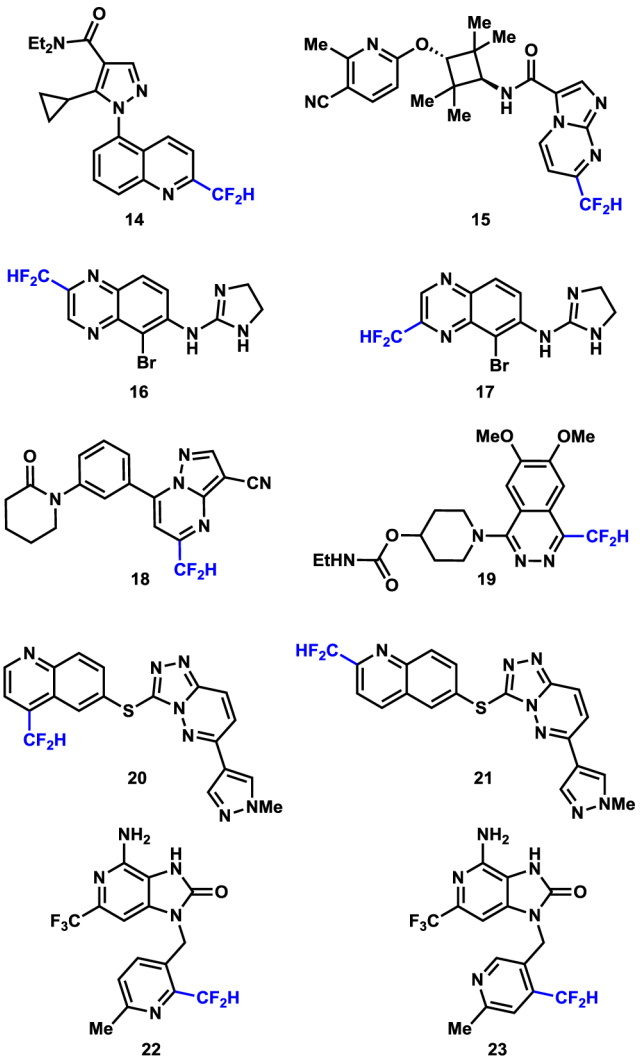

Figure 2. Metabolically blocked difluoromethyl analogues show resistance to AO. Difluoromethylated products isolated from larger scale reactions showed resistance to AO activity in all cases except **20**. Reactions were carried out at 0.125 or 0.25 mmol scale and isolated by preparative HPLC. Some compounds isolated as TFA salts (see Supporting Information).

previous studies have shown that difluoromethylation of a heteroarene appeared to retard difluoromethylation at other functionalizable sites, such that it was usually possible to avoid disubstituted products. This is illustrated in the LCMS traces in Tables 1 and 2, where disubstituted products are rarely seen under conditions where multiple different difluoromethylated isomers may be formed.[17] Intriguingly, in **3**, which has two possible sites that may be metabolized by AO, difluoromethylation of any one site prevented oxidation at the other. This may be partly attributed to steric effects, but a clear example of the effect that electronic tuning may have on AO activity is seen when comparing **22** and **23**. While it may be expected that **22** will not be an AO substrate as the oxidized position adjacent to nitrogen is blocked, the resistance of **23** to AO activity is unexpected and suggests that the additional regioisomers produced by DFMS substitution may be of value when testing for enhanced metabolic profiles. However, this electronic effect may not be general, for example, **21** showed resistance to AO but its isomer **20** proved to be an AO substrate.

CONCLUSION

In summary, a simple "litmus test" procedure using the nucleophilic radical source DFMS has been developed to alert medicinal chemists of the likelihood that azaheteroaromatic drug candidates may be substrates for AO. The results can inform decisions about early biological AO testing. The litmus test also produces metabolically blocked analogues for further testing. Finally, this work points to an unusual case where a chemical reaction can rapidly decipher subtle information about the innate reactivity of a drug candidate, thereby giving meaningful data on a potentially devastating metabolic liability.

EXPERIMENTAL SECTION

Compounds **14**–**23** were synthesized as detailed in the representative example below. The purity of all final compounds was determined to be ≥95% by NMR and LCMS analysis. General methods, full experimental details and original spectra may be found in the Supporting Information.

1-(4-(Difluoromethyl)-6,7-dimethoxyphthalazin-1-yl)-piperidin-4-yl ethylcarbamate (19). TFA (10 μL) was added to a solution of 1-(6,7-dimethoxyphthalazin-1-yl)piperidin-4-yl ethylcarbamate (**4**) (45 mg) and DFMS (74 mg) in DMSO (0.7 mL) in a small screw-capped vial. The reaction was cooled in ice as TBHP (70% aq solution) (52 μL) was added slowly to the stirred solution. The vial was sealed and stirred at 50 °C for 2 h. The reaction mixture was cooled to RT, transferred to a separating funnel (rinsing with EtOAc), and diluted with 10 mL of EDTA/sodium bicarbonate solution (prepared by dissolving 18 g of EDTA disodium salt in 150 mL of saturated sodium bicarbonate solution). The aqueous solution was extracted into EtOAc (2 × 10 mL), and the combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by preparative HPLC to give 16 mg of **19** TFA salt as a cream-colored powder (24% yield); mp 192–196 °C; R_f = 0.67 (100% EtOAc). ^{1}H NMR (600 MHz, MeOD) δ 7.59 (s, 1H), 7.40 (s, 1H), 7.05 (t, J = 53.8 Hz, 1H), 4.98 − 4.90 (m, 1H), 4.06 (s, 3H), 4.03 (s, 3H), 3.76–3.58 (m, 2H), 3.50–3.38 (m, 2H), 3.15 (q, J = 7.2 Hz, 2H), 2.28–2.13 (m, 2H), 2.03–1.91 (m, 2H), 1.12 (t, J = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 162.6, 158.3, 155.5, 155.2, 147.8 (t, J = 27.0 Hz), 123.1, 119.7, 118.7 (t, J = 238.8 Hz), 104.4, 104.0 (t, J = 3.2 Hz), 71.0, 56.7, 56.7, 36.5, 32.2, 15.4. ^{19}F NMR (376 MHz, MeOD) δ −77.0, −114.1. IR (solid) ν = 3276, 2929, 2834, 1698, 1608, 1516, 1453, 1427, 1245, 1214, 1024, 841 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{19}H_{24}F_2N_2O_4H^+$ [M + H$^+$], 411.1838; found, 411.1843.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data for all new compounds, including ^{1}H, ^{13}C, and ^{19}F NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The crystal structure image of AO used in our table of contents graphic was created from data submitted by ref 7a to the Protein Data Bank. PDB ID: 3ZYV.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AO, aldehyde oxidase; C−H, carbon−hydrogen; CYP, cytochrome P450; DFMS, bis(((difluoromethyl)sulfinyl)oxy)zinc; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; LCMS, liquid chromatography mass spectrometry; MeOH, methanol; MW, molecular weight; NMR, nuclear magnetic resonance; RT, room temperature; TBHP, *tert*-butyl hydroperoxide; TFA, trifluoroacetic acid; TIC, total ion current

■ REFERENCES

(1) (a) Gleeson, M. P.; Hersey, A.; Montanari, D.; Overington, J. Probing the links between in vitro potency, ADMET and physicochemical parameters. *Nature Rev. Drug. Discovery* **2011**, *10*, 197−208. (b) Leeson, P. D.; Empfield, J. R. Reducing the risk of drug attrition associated with physicochemical properties. *Annu. Rep. Med. Chem.* **2010**, *4*, 393−407.

(2) (a) Pryde, D. C.; Dalvie, D.; Hu, Q.; Jones, P.; Obach, R. S.; Tran, T.-D. Aldehyde oxidase: an enzyme of emerging importance in drug discovery. *J. Med. Chem.* **2010**, *53*, 8441−8460. (b) Garattini, E.; Terao, M. Increasing recognition of the importance of aldehyde oxidase in drug development and discovery. *Drug. Metab. Rev.* **2011**, *43*, 374−386. (c) Garattini, E.; Terao, M. The role of aldehyde oxidase in drug metabolism. *Expert Opin. Drug Metab. Toxicol.* **2012**, *8*, 487−503. (d) Garattini, E.; Terao, M. Aldehyde oxidase and its importance in novel drug discovery: present and future challenges. *Expert Opin. Drug Discovery* **2013**, *8*, 641−654. (e) St. Jean, D. J.; Fotsch, C. Mitigating heterocycle metabolism in drug discovery. *J. Med. Chem.* **2012**, *55*, 6002−6020. (f) Hutzler, J. M.; Obach, R. S.; Dalvie, D.; Zientek, M. A. Strategies for a comprehensive understanding of metabolism by aldehyde oxidase. *Expert Opin. Drug Metab. Toxicol.* **2013**, *9*, 153−168.

(3) Kaye, B.; Rance, D. J.; Waring, L. Oxidative metabolism of carbazeran in vitro by liver cytosol of baboon and man. *Xenobiotica* **1985**, *15*, 237−242.

(4) Diamond, S.; Boer, J.; Maduskuie, T. P.; Falahatpisheh, N.; Li, Y.; Yeleswaram, S. Species-specific metabolism of SGX523 by aldehyde oxidase and the toxicological implications. *Drug Metab. Dispos.* **2010**, *38*, 1277−1285.

(5) See the following articles and references therein: (a) Lewis, D. F. V. On the recognition of mammalian microsomal cytochrome P450 substrates and their characteristics: towards the prediction of human p450 substrate specificity and metabolism. *Biochem. Pharmacol.* **2000**, *60*, 293−306. (b) de Groot, M. J. Designing better drugs: predicting cytochrome P450 metabolism. *Drug Discovery Today* **2006**, *11*, 601−606. (c) Ekins, S.; de Groot, M. J.; Jones, J. P. Pharmacophore and three-dimensional quantitative structure−activity relationship methods for modeling cytochrome P450 active sites. *Drug Metab. Dispos.* **2001**, *29*, 936−944. (d) Cruciani, G.; Carosati, E.; De Boeck, B.; Ethirajulu, K.; Mackie, C.; Howe, T.; Vianello, R. MetaSite: understanding metabolism in human cytochromes from the perspective of the chemist. *J. Med. Chem.* **2005**, *48*, 6970−6979.

(6) There have been several reported approaches to predicting site of AO oxidation, or predicting AO clearance for known AO substrates, but none of these can predict whether or not a compound will be an AO substrate. Two separate groups have developed computational models that are able to accurately predict which position on a known substrate will be most prone to oxidation: (a) Torres, R. A.; Korzekwa, K. R.; McMasters, D. R.; Fandozzi, C. M.; Jones, J. P. Use of density functional calculations to predict the regioselectivity of drugs and molecules metabolized by aldehyde oxidase. *J. Med. Chem.* **2007**, *50*, 4642−4647. (b) Dastmalchi, S.; Hamzeh-Mivehrod, M. Molecular modelling of human aldehyde oxidase and the identification of the key interactions in the enzyme−substrate complex. *Daru, J. Fac. Pharm., Tehran Univ. Med. Sci.* **2005**, *13*, 82−93. A limited study has correlated the site of amination of 1-alkyl-3-carbamoylpyridinium chlorides in liquid ammonia with the site of AO oxidation (c) Angelino, S. A. G. F.; van Veldhuizen, A.; Buurman, D. J.; van der Plas, H. C. Covalent amination of 1-alkyl- and 1-aryl-3-carbamoylpyridinium chlorides as "model" for enzymic activity of rabbit liver aldehyde oxidase. *Tetrahedron* **1984**, *40*, 433−439. A computational model is able to predict AO clearance more effectively than animal models (d) Jones, J. P.; Korzekwa, K. R. Predicting intrinsic clearance for drugs and drug candidates metabolized by aldehyde oxidase. *Mol. Pharmaceutics* **2013**, *10*, 1262−1268.

(7) (a) Coelho, C.; Mahro, M.; Trincão, J.; Carvalho, A. T. P.; Ramos, M. J.; Terao, M.; Garattini, E.; Leimkühler, S.; Romão, M. The first mammalian aldehyde oxidase crystal structure: insights into substrate specificity. *J. Biol. Chem.* **2012**, *287*, 40690−40702. (b) Kitamura, S.; Sugihara, K.; Ohta, S. Drug-metabolizing ability of molybdenum hydroxylases. *Drug Metab. Pharmacokinet.* **2006**, *21*, 83−98.

(8) Alfaro, J. F.; Jones, J. P. Studies on the mechanism of aldehyde oxidase and xanthine oxidase. *J. Org. Chem.* **2008**, *73*, 9469−9472.

(9) Pryde, D. C.; Tran, T.-D.; Jones, P.; Duckworth, J.; Howard, M.; Gardner, I.; Hyland, R.; Webster, R.; Wenham, T.; Bagal, S.; Omoto, K.; Schneider, R. P.; Lin, J. Medicinal chemistry approaches to avoid aldehyde oxidase metabolism. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2856−2860.

(10) O'Hara, F.; Blackmond, D. G.; Baran, P. S. Radical-based regioselective C−H functionalization of electron-deficient heteroarenes: scope, tunability, and predictability. *J. Am. Chem. Soc.* **2013**, *135*, 12122−12134.

(11) Fujiwara, Y.; Dixon, J. A.; Rodriguez, R. A.; Baxter, R. D.; Dixon, D. D.; Collins, M. R.; Blackmond, D. G.; Baran, P. S. A new reagent for direct difluoromethylation. *J. Am. Chem. Soc.* **2012**, *134*, 1494−1497. Bis(((difluoromethyl)sulfinyl)oxy)zinc (DFMS) is available from Sigma-Aldrich; catalogue no. 767840.

(12) Dalvie, D.; Sun, H.; Xiang, C.; Hu, Q.; Jiang, Y.; Kang, P. Effect of structural variation on aldehyde oxidase-catalyzed oxidation of zoniporide. *Drug Metab. Dispos.* **2012**, *40*, 1575−1587.

(13) Linton, A.; Kang, P.; Ornelas, M.; Kephart, S.; Hu, Q.; Pairish, M.; Jiang, Y.; Guo, C. Systematic structure modifications of imidazo[1,2-*a*]pyrimidine to reduce metabolism mediated by aldehyde oxidase (AO). *J. Med. Chem.* **2011**, *54*, 7705−7712.

(14) Ghafourian, T.; Rashidi, M. R. Quantitative study of the structural requirements of phthalazine/quinazoline derivatives for interaction with human liver aldehyde oxidase. *Chem. Pharm. Bull.* **2001**, *49*, 1066−1071.

(15) For an example of a similar ketolide antibiotic where susceptibility to AO did correlate with a positive DFMS test, see compound **SI-2** in the Supporting Information.

(16) Magee, T. V.; Ripp, S. L.; Li, B.; Buzon, R. A.; Chupak, L.; Dougherty, T. J.; Finegan, S. M.; Girard, D.; Hagen, A. E.; Falcone, M. J.; Farley, K. A.; Granskog, K.; Hardink, J. R.; Huband, M. D.; Kamicker, B. J.; Kaneko, T.; Knickerbocker, M. J.; Liras, J. L.; Marra, A.; Medina, I.; Nguyen, T.-T.; Noe, M. C.; Obach, R. S.; O'Donnell, J. P.; Penzien, J. B.; Reilly, U. D.; Schafer, J. R.; Shen, Y.; Stone, G. G.; Strelevitz, T. J.; Sun, J.; Tait-Kamradt, A.; Vaz, A. D. N.; Whipple, D. A.; Widlicka, D. W.; Wishka, D. G.; Wolkowski, J. P.; Flanagan, M. E. Discovery of azetidinyl ketolides for the treatment of susceptible and multidrug resistant community-acquired respiratory tract infections. *J. Med. Chem.* **2009**, *52*, 7446−7457.

(17) Multiple substitutions may be more commonly seen in substrates such as **6**, which has multiple electronically distinct heteroaromatic rings.