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Complementation of Biotransformations with Chemical C−H Oxidation: Copper-Catalyzed Oxidation of Tertiary Amines in Complex Pharmaceuticals

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Supporting Information

ABSTRACT: The isolation, quantitation, and characterization of drug metabolites in biological fluids remain challenging. Rapid access to oxidized drugs could facilitate metabolite identification and enable early pharmacology and toxicity studies. Herein, we compared biotransformations to classical and new chemical C−H oxidation methods using oxcarbazepine, naproxen, and an early compound hit (phthalazine **1**). These studies illustrated the low preparative efficacy of biotransformations and the inability of chemical methods to oxidize complex pharmaceuticals. We also disclose an aerobic catalytic protocole (CuI/air) to oxidize tertiary amines and benzylic CH's in drugs. The reaction tolerates a broad range of functionalities and displays a high level of chemoselectivity, which is not generally explained by the strength of the C−H bonds but by the individual structural chemotype. This study represents a first step toward establishing a chemical toolkit (chemotransformations) that can selectively oxidize C−H bonds in complex pharmaceuticals and rapidly deliver drug metabolites.

INTRODUCTION

The development of functional cytochromes P450[1] (CYP)-mimetic reactions has been a long-standing goal in organic chemistry. Over the years, many catalytic systems such as metalloporphyrins and related systems have been discovered.[2a] Yet, their applications have been constrained to simple or specific classes of substrates.[2b,c] With the exception of the Udenfriend system,[3] a classical Fe-mediated hydroxylation of arenes, none of these methods have found wide applications for the synthesis and identification of drug metabolites (MetID).

It is well-known that the metabolism of drugs can produce metabolites with profoundly altered functional parameters such as biological activity, clearance rates, and toxicity.[4] Thus, tools that facilitate the rapid identification and synthesis on scale of drug metabolites hold a great potential to facilitate the drug discovery cycle and guide the selection of clinical candidates. MetID is traditionally accomplished via analysis of complex biological matrices with liquid chromatography (LC)/mass spectrometry (MS/MS). It mainly provides qualitative information on product distribution as analytical references are not readily accessible. In most cases, unambiguous structural elucidation is hampered by difficulties associated with compound fragmentation in MS. In addition, metabolites cannot be easily isolated on scale to enable either structural elucidation or biological evaluation. To circumvent some of these issues, biotransformation approaches have been developed and are routinely used to scale up metabolites in drug discovery and provide references for metabolite detection in clinic, although challenges still remain.[5] In humans, nearly 57 CYP isoforms are involved in the metabolism of drugs;[6] many of these are not currently available in recombinant form for biotransformation purposes. Moreover, the process yields a broad range of metabolites, often formed in trace quantities. This limits access to practical quantities of these compounds required for structural elucidation and further studies. *Consequently, we became interested in developing a comprehensive set of chemical reactions, termed here as chemotransformations, that can be used to access putative metabolites, guided by the current knowledge of CYP reactions (e.g., MetaSite, Scheme 1a).*[7]

At the outset, we hypothesized that modern C−H oxidation reactions could complement CYP-mediated biotransformations, thus expanding the range of drug oxidation products that can be rapidly accessed.[8] Guided by this premise, we perfomed a comparative study using phthalazine (**1**), an internal hit that inhibits the smoothened Hedgehog (Hh) signaling pathway (Table 1),[9] oxcarbazepine, a generic anticonvulsant and mood stabilizing drug,[10] and naproxen, a nonsteroidal anti-inflammatory drug (see the Supporting Information (SI)).[11] These substrates were selected to reflect the most common types of CYP-mediated metabolic reactions guided by either MetaSite predictions[7] or the reported literature on their clinical

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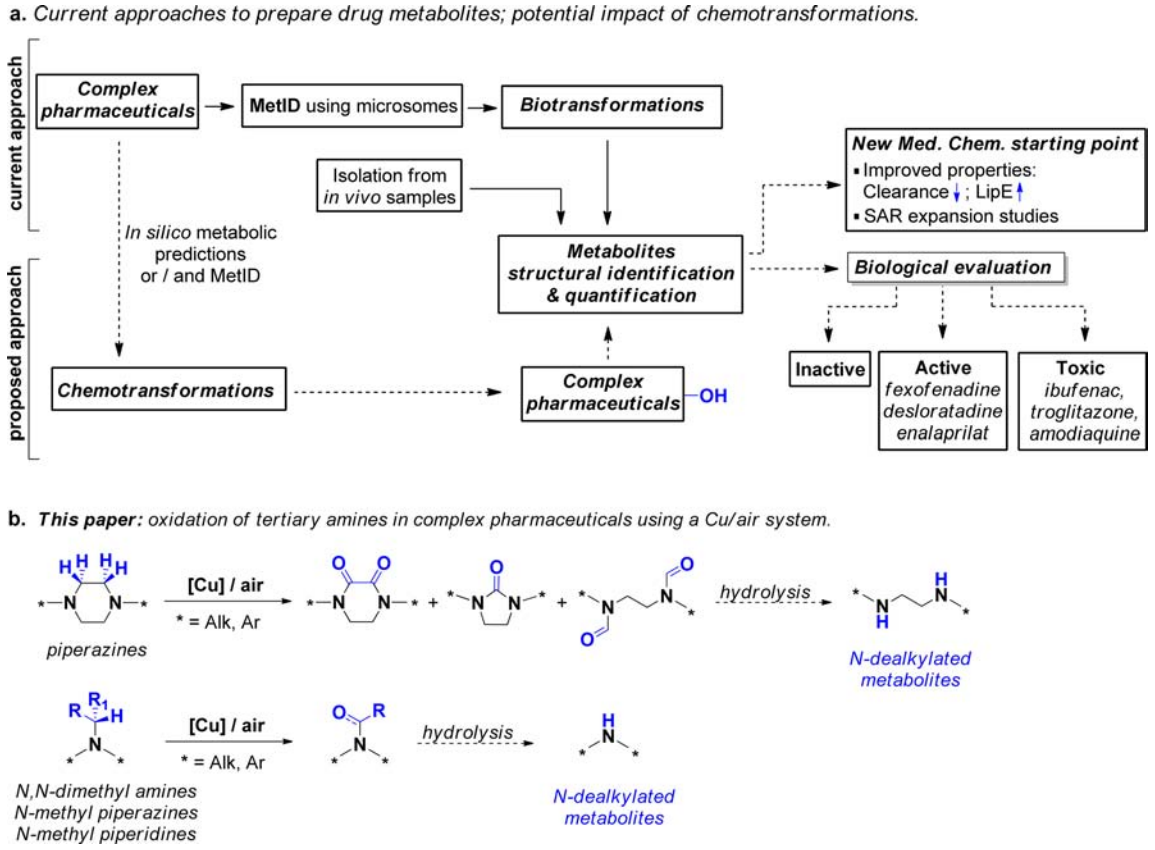
Scheme 1. Preparation of Drug Metabolites: Current Status and Potential Future Directions



metabolites or both. We found that chemical C−H oxidation methods were ineffective in oxidizing complex pharmaceuticals and providing access to putative drug metabolites.

Herein, we show that a simple CuI/air catalytic system[12] mimics several aspects of CYP reactivity toward tertiary amines and a subset of benzylic C−H bonds in complex pharmaceuticals. It affords drug metabolites or their precursors (*N*-carbonyl compounds) in preparative quantities (Scheme 1b). It is worth noting that benzylic groups and tertiary alkylated amines are ubiquitous structural features found in drugs, compound hits, and leads.[13,14] The benzyl groups are readily metabolized by CYPs to form benzylic alcohols, which can be further oxidized into ketones and carboxylic acids, whereas tertiary amines afford amides/lactams and *N*-dealkylated metabolites.[15] Currently, chemical catalytic systems that mimic the CYP-catalyzed reactions are rare despite the activated nature of these C−H bonds. Indeed, catalytic processes that convert tertiary amines to the corresponding amides/lactams are uncommon.[16,17]

The broader use of this method is predicated upon an understanding of the chemoselectivity of the reaction. The use of simplified substrates to better understand the reactivity of this new catalytic system and gain some insight into the reaction mechanisms is also discussed.

RESULTS AND DISCUSSION

Bio- and Chemotransformations Comparative Study. For the preparative scale synthesis of drug oxidation products, the following systems were used: 10 S9 fractions (i.e., liver homogenates that contain both the microsomes and the cytosol of hepatocytes) from 10 different species; *E. coli* JM109 whole-cells recombinantly expressing 1 out of 14 human CYP isoenzymes (rhCYP) together with human CYP reductase, and 5 microbial CYP.[18] It is important to note that these catalyst systems were used under non physiological conditions and thus the results obtained in this study differ from those acquired in CYP isotyping or microsomal incubation studies and do not reflect the in vivo metabolism.

After an analytical screening using the noted set of biocatalysts to identify active isoforms, the reactions were scaled up for HPLC purification and NMR structural elucidation investigations.[18] Phthalazine **1** was extensively oxidized (Table 1); nine biotransformation products were detected in the analytical screening using recombinant enzymes (Table 6, SI). Additional metabolites could also be observed using S9 fractions (Table 7, SI). Monohydroxylation (**2**, 35.7%; **3**, 55.4%), bis-hydroxylation (**4**, 11.7%; **5**, 4.3%; **6**, 4.3%; **7**, 11.0%), and tris-hydroxylation (**8**, 5.9%) occurred in various regions of the molecule in individual runs (details provided in Table 1). In addition to the biotransformation products enumerated above, which could not be characterized by simple LC-MS/MS, the cleaved piperazine ring derivative **9** (9.8%) and dehydrated parent **10** (7.6%) were also observed. The vast majority of these biotransformation products could be produced using rhCYP3A4b5 and bacterial CYP102A1. The former was chosen for scale up to support NMR characterization studies of **2**-**8** (30 mg scale biooxidation).[19] Five biotransformation products were isolated and identified by NMR (Table 1). MetaSite predicted benzylic oxidation products **11** (32%) and **12** (7%) as the major products of the reaction. Para-aromatic hydroxylation of the phenyl ring was also observed (**13**, 0.6%) forecasting potential for quinone

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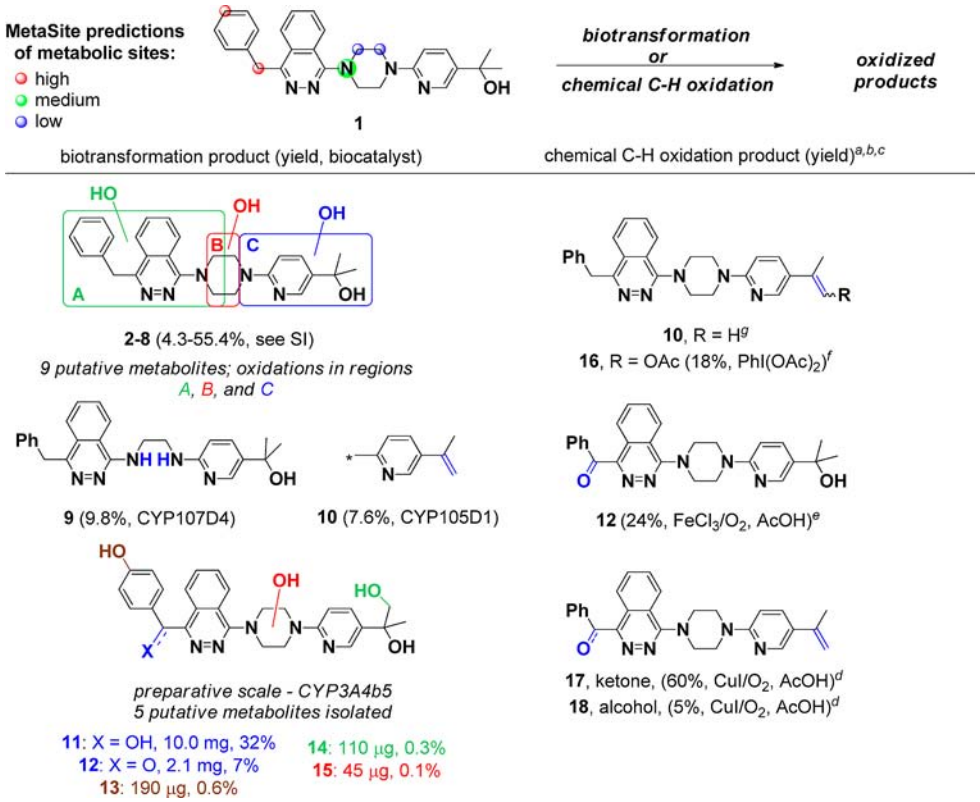
Table 1. Comparative Study between Bio- and Chemotransformations



[a]Asterisk (*) indicates that, when the structures are too large, only reaction centers are shown; the structures of **11**–**15** share the backbone in black; individual reaction products are labeled by color. [b]Average of at least two isolated yields. [c]Chemical conditions explored (see the SI). [d]CuI (10 mol %), O_2, AcOH (1 equiv.), DMSO, 110 °C, 16 h. [e]$FeCl_3$ (10 mol %), O_2, AcOH (1 equiv.), DMSO, 110 °C, 16 h. [f]$PhI(OAc)_2$ (2 equiv.), AcOH, 100 °C, 2–16 h. [g]The tertiary alcohol proved labile under most reaction condition.

methide formation in vivo. sp^3 hydroxylation took place on both the gem-dimethyl adjacent to the pyridine ring (**14**, 0.3%) and at the piperazine ring (**15**, 0.1%). In summary, all of the putative metabolites of **1** were obtained via biotransformations (**9**, **11**, **12**, and **13**; Table 1) along with many other oxygenated products that were not predicted by MetaSite to be metabolites. However, in many cases, the products were obtained in low yield, thus posing significant isolation and structural characterization challenges. In addition, for naproxen and oxcarbazepine, the process was inefficient altogether (see the SI), thus providing the impetus to develop alternative strategies such as chemical means to access metabolites on scale. Toward this goal, a diverse set of modern sp^2/sp^3 C–H oxidation conditions[20] developed by several research teams were investigated, including those employing copper,[20b] gold,[20c] iridium,[20d,e] iron,[2b,3a] manganese,[20f] palladium,[20g–i] platinum,[20j] ruthenium,[20k] rhenium,[20l] silver,[20m] and nonmetallic catalysts.[20n] We were mindful of the fact that, in most cases, the substrates selected for this study did not match the reported substrate scope of the catalysts, but these reactions represented the latest advances in the field. Most conditions applied to phthalazine **1** led to the formation of *N*-oxides, no reaction, or complete decomposition. The only identifiable product was the formation of vinyl acetate **16** under $Pd/PhI(OAc)_2$ conditions, which incidentally did not require the presence of the metal.

The results discussed above served to illustrate the inability of the classical and recently developed C–H oxidation methods to effect oxidation of complex pharmaceutical compounds. Further advances in this arena would require the development of new catalytic reactions that tolerate common structural features of drugs such as the presence of basic nitrogen.

We set the modest goal of accessing **11** and **12** (Table 1) using copper and molecular oxygen based on exploratory benzylic oxidations recently disclosed,[21] which focused on simple substrates.[22] In the presence of a catalytic amount of CuI (10 mol %), O_2, and AcOH (1 equiv.) at 110 °C in DMSO, **1** underwent oxidation of the benzylic methylene position concomitant with loss of the tertiary alcohol (a facile process that was noted under many previous C–H oxidation reactions) to furnish **17** in excellent yield (60% isolated yield; Table 1). Alcohol **18** was also recovered in 5% isolated yield. The use of $FeCl_3$ (10 mol %) in lieu of CuI led to the direct formation of the penultimate product **12** (24%), which may be reduced to give **11**. These successes prompted us to further probe the usefulness of this simple CuI/air catalytic system for metabolites synthesis, and these efforts are discussed below.

Optimization and Scope Examination of the New C–H Oxidation Methodology. We began with chloropheniramine **19**, a first-generation alkylamine antihistamine, which featured a tertiary heterobenzylic C–H bond; an unknown substrate for this catalytic system (Table 2, entry 1). Unexpectedly, formamide **20** and ketone **21** were formed in a 2:1 ratio and a combined 39% yield under the reaction conditions. The formation of **21** can be rationalized by first oxidizing at the benzylic position to form a tertiary γ-amino-alcohol in situ, which then underwent a fragmentation reaction via extrusion of ethylene and dimethylamine to afford the title compound. The formation of metabolite precursor **20** defines

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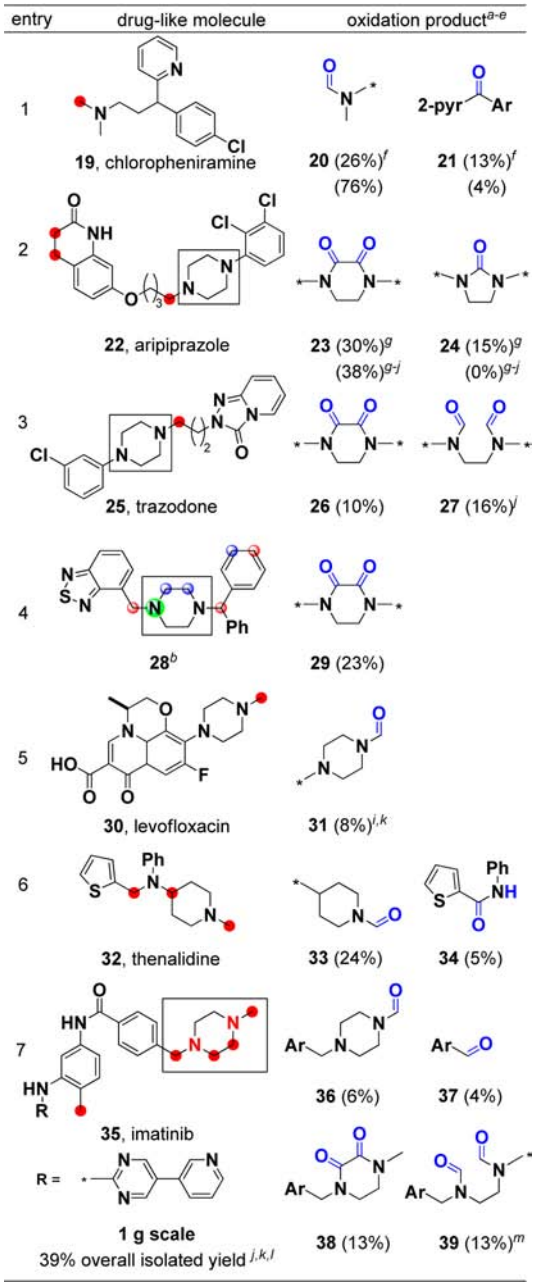
Table 2. Scope of the Copper-Catalyzed C−H Oxidation of Generic Drugs



[a]Red dots indicate sites of metabolism in vivo. [b]MetaSite predictions were used for **28** since human metabolites were not known (see Table 1 for color coding). [c]Asterisk (*) indicates that only reaction centers are shown. [d]Condition: drug 0.1 M, CuI (20 mol %), air or O_2 (balloon), DMSO, 120 °C, 16 h. [e]Average of at least 2 isolated yields. [f]Conditions as in *d*, plus AcOH (1 equiv.). [g]30% of parent drug recovered. [h]Conditions as in *d*, plus TEMPO (2 equiv.). [i]CuI (1 equiv.). [j]See the SI. [k]Successive purifications account for the modest yields. [l]Urea was also isolated (3%). [m]^{1}H NMR yield.

another new catalytic reaction. Its yield could be improved to 76% with only minimal formation of **21** (4%) by running the reaction at 120 °C and excluding acetic acid from the reaction mixture. This process, if broad in scope, could constitute a viable route to access common amine-containing drug metabolism (formation of amides/lactams and *N*-dealkylated) products.[23] Also, amines are associated with hERG liabilities and these are often addressed by decreasing their pK_a via amidation and *β*-fluorination. Thus, amine dealkylation or their direct conversion into amides will find applications in chemotransformations and in medicinal chemistry in general.

With this goal in mind, we first selected a series of drugs containing privileged amines in pharmaceuticals such as piperazine, 4-amino piperidine, and *N*-methyl amine motifs. The known or predicted sites of metabolism are highlighted (Table 2). We started with aripiprazole **22**, a new generation antipsychotic, which features differentially substituted amines in the piperazine ring. When submitted to our reaction conditions (no AcOH), the piperazine ring was selectively converted into 2,3-diketopiperazine (DKP) **23** and 5-membered urea **24** in 45% overall yield (Table 2, entry 2). Increasing catalyst loading (0.2 or 1 equiv.) or the reaction temperature (120 or 150 °C) did not significantly improve the conversion of the drug. Given the propensity of copper reagents to generate radicals, we added TEMPO to the reaction mixture to see how it would affect product yield and distribution. We noted complete suppression of compound **24** and the exclusive formation of **23** in 38% isolated yield. An analogous differentially substituted piperazine, trazodone **25**, a triazolopyridine antidepressant, was also oxidized into DKP **26** (10%) along with 16% of a formal C−C cleavage product (bis-formamide **27**, entry 3). The formation of the 5-membered urea (vide supra) and other piperazine fragmentation products is the subject of mechanistic investigations (see below). Moving to *N*,*N*′-dialkyl piperazine **28** (entry 4), DKP **29** was isolated as the major component of the crude mixture in 23% yield. Here, no significant piperazine fragmentation byproducts were formed and no benzylic C−H's were oxidized. Moreover, no sulfur oxidation product was isolated. In the case of levofloxacin **30**, a fluoroquinolone antibiotic, only *N*-formyl product **31** was isolated in 8% (entry 5), despite full and reasonably clean conversion to the product, due to product instability during purification. When the piperazine ring was replaced by 4-aminopiperidine, such as in thenalidine **32**, an antihistamine/anticholinergic drug, the exocyclic *N*-methyl C−H's became the preferred target of the catalyst (formamide **33** was isolated in 24%, entry 6). The minor product of this reaction (**34**, 5%) resulted from C−N bond cleavage of the *N*-alkyl aniline and the oxidation of the methylene adjacent to thiophene. It is important to note that the electron-rich thiophene ring was left mostly intact. Finally, to demonstrate the scalability of this methodology, imatinib **35**, a flagship Novartis oncology drug, was oxidized on 1 g scale in an overall 39% isolated yield to give a product distribution consistent with the selectivity preferences examined earlier (Table 2). Oxidation of both exocyclic C−H bonds (benzylic and *N*-methyl positions) occurred as minor reaction pathways leading to the formation of the corresponding formamide **36** and benzaldehyde **37**. In contrast, endocyclic C−H's were oxidized to form the major products: DKP **38**, bis-formamide **39**, and 5-membered urea (not shown, see SI). In all cases, sufficient material was obtained to enable in vitro biological evaluation of the products and PK studies. For substrates **19**, **30**, **32**, and **35**, similar to CYP, the catalyst selectively targeted the major metabolism soft spots, affording activated compounds that could be converted in one step into *N*-dealkylation metabolites. Remarkably, the vast majority of imatinib metabolism soft spots were all targeted in a single step,[24] and this illustrates the power of the approach described herein. In other instances (**22**, **25**, and **28**), oxidation products did not correspond to known metabolites; however, they reflect

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common occurrences in CYP-mediated piperazine metabolism. Indeed DKPs are known metabolites,[25] and in addition, ring-opening of ureas should afford piperazine ring fragmentation amine metabolites.

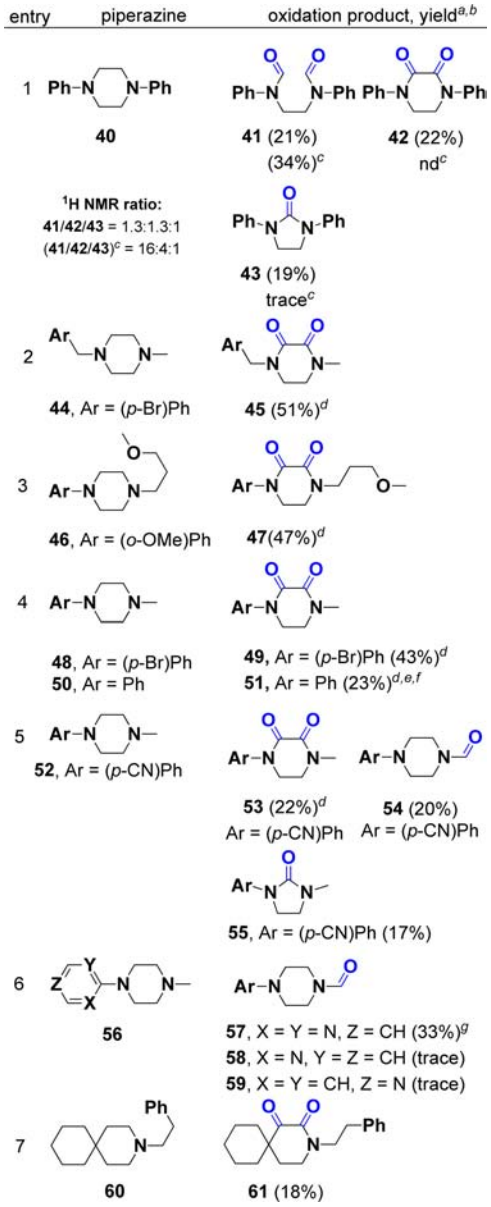
Collectively, these studies demonstrate that the selective functionalization of tertiary amines in complex pharmaceuticals to afford products that are drug metabolites or their precursors is possible using a very simple copper salt/air catalytic system. Although copper was reported to oxidize benzylic methylenes of simple arenes, the chemoselective copper-mediated oxidation of complex pharmaceuticals to generate drug metabolites is unprecedented.

With the exception of **30**, when piperazines were embedded within the substrates, they were selectively converted into DKPs, bis-formamides, or ureas. At this juncture, the remaining key issues were a better understanding of reactivity patterns observed above and the mechanisms of the reaction. These studies required the use of simplified substrates discussed next.

To gain some understanding on the chemoselectivity and the predictability of the method, a series of simplified substrates were submitted to the reaction condition starting with 1,4-diphenylpiperazine (Table 3, entry 1). Here, piperazine ring-fragmented bis-formamide (**41**), DKP (**42**), and ring-contracted urea products (**43**) were obtained in a combined 62% isolated yield and 1.3:1.3:1 ratios. The addition of TEMPO to the reaction mixture produced bis-formamide **41** selectively (34%). When 1,4-dialkyl piperazine **44** or 1-aryl-4-alkyl piperazines, **46**, **48**, and **50**, were used as substrates (entry 3 and 4), no ring fragmentation products were observed. In all cases, the corresponding DKPs were obtained in 22−51% yield as the major products of the reaction. Of note, direct synthesis of DKPs from piperazines is rare.[26] This motif has found widespread applications in drug design[27,28] and is also encountered in natural products.[29] Interestingly, placing a cyano-group at a distal position on the arene (**52**, entry 5) altered the product distribution profile; oxidation of the methyl group into formamide **54** was significant relative to the oxidation of endocyclic piperazine C−H bonds. The cyclic urea product was also observed in higher yield (**55**, 17% isolated). Replacing the benzonitrile moiety with a pyrimidine (entry 6) completely shielded the piperazine C−H bonds against oxidation; yielding instead *N*-formamide product **57** in 33% yield. In some ways this system is similar to CYP which prefers electron-rich positions. Under the reaction conditions, **58** and **59** were only formed as trace amounts due to poor conversion and decomposition to a range of unidentified products. Finally, piperidine **60** was oxidized at both the α and β positions of the amine yielding a product that would be very difficult to access otherwise; again no mono-oxidation product was observed.

In summary, the oxidation of the piperazine ring was facile and was preferred even in the presence of a primary *N*-methyl group, affording DKPs. The exceptions were *N*-pyrimidinyl (Table 3, entry 6) and the *N*-phthalazinyl (Table 1) substrates suggesting that electron-deficient heteroarene rings shield the piperazine from oxidation. In contrast to more complex substrates (Table 2), ring fragmentation occurred only when both nitrogens of the piperazine ring were arylated. Regardless of the complexity of the substrates (Table 2, entry 2, versus Table 3, entry 1), addition of TEMPO to the reaction mixture precluded the formation of 5-membered urea. The role of TEMPO as well as the ligand (DMSO) will be investigated in the future studies.

Table 3. Oxidation of *N,N′* Disubstituted Piperazines



[a]Reaction condition: CuI (20 mol %), air or O_2 balloon, DMSO, 120 °C, 16 h. [b]Average of at least 2 isolated yields. [c]TEMPO (2 equiv.) was added. [d]Trace of pyrazine-2,3(1*H*,4*H*)-dione was observed. [e]Trace of formamide was observed. [f]Trace of cyclic urea was observed. [g]48% starting material recovered.

Mechanistic Investigation. To understand the formation of formamides and cyclic ureas under the current reaction conditions, 2,3-DKP **42** was submitted to the reaction conditions (Scheme 2). The compound proved to be stable with no noticeable formation of urea or *N,N′*-bis-formamide, thus excluding it as a putative intermediate. We then speculated that products such as **41** and **43** may be formed from a diamine ring-opened product (double C−N cleavage). To probe this possibility, commercially available *N,N′*-diphenylethylenediamine (**62**) was submitted to the reaction conditions (Scheme 2). The formation of mono- formamide **63** was observed. Upon addition of more Cu-catalyst and heating, it was further converted into urea **43** and *N,N′*-bis-formamide **41** along with variable amounts of aniline **64**. Although, further experiments

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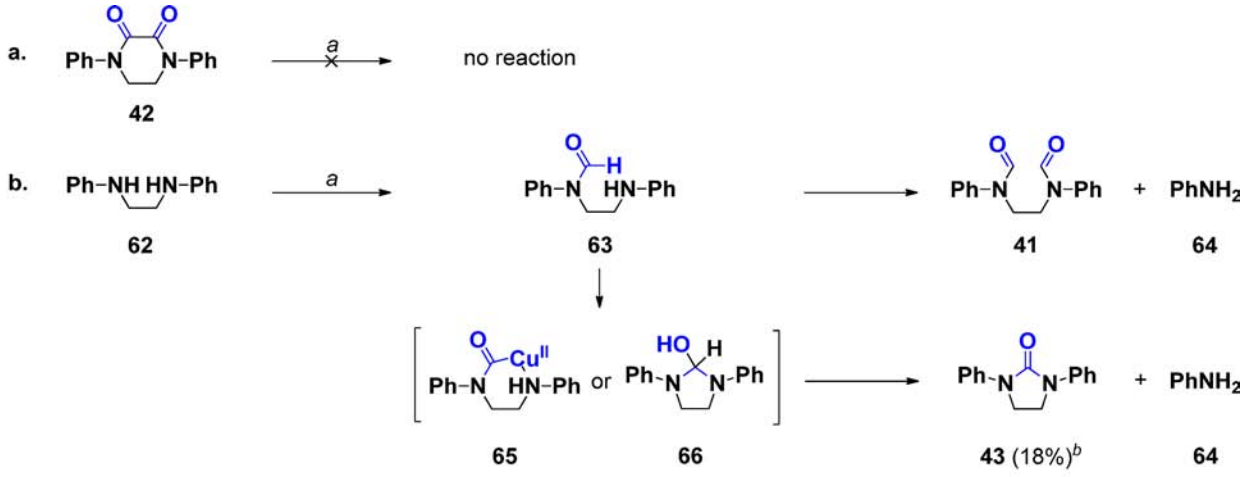
Scheme 2. Probing the Mechanism of Formation of Ring-Opened and Ring-Contracted Products Derived from Piperazines



[a] Condition as in Table 3; [b] isolated yield

are needed to determine the intimate mechanistic details of this transformation, the above results suggested that the piperazine ring itself or DMSO was the source of the carbonyl/formyl group, and C−N bond cleavage to form diamines takes place under the reaction conditions. The latter intermediate then underwent two *N*-formylations to form a bis-formamide (such as **41**) or one *N*-formylation, followed by activation of the formyl C−H bond with subsequent C−N coupling or oxidative ring closure (intermediate **65** or **66**) to afford a urea (such as **43**). We also demonstrated that molecular oxygen acted as the oxygen donor by adding ^{18}O-labeled water to the reaction mixture, and the incorporation of ^{18}O in the product was not observed when using 1,4-diphenyl piperazine. Finally, pure isolated bis-formamide **41** and urea **43** were separately also resubmitted to the reaction conditions; none of these substrates reacted even at 150 °C over 24 h confirming they are end products and not intermediates toward the formation of one another.

CONCLUSION

A simple copper/molecular oxygen (or air) system has uncovered a broad range of novel reactivity that can be used with complex pharmaceuticals to access drug metabolites, their precursors, improved new drug candidates, or intermediates for further SAR studies. We anticipate that this operationally simple ligand-free catalyst system, will find many applications in both metabolism studies and synthesis. Broadly, the use of simple metal salts and air to study the reactivity of C−H's and deduce general reactivity principles that can guide metabolite identification and synthesis on scale holds significant promise and is the focus of current and future studies in our laboratories.

ASSOCIATED CONTENT

Supporting Information

Detailed procedure for the bio- and chemical transformations, HPLC/LC-MS methods, and data such as NMR spectra of isolated products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Ortiz de Montellano, P. R.; De Voss, J. J. *Substrate Oxidation by Cytochrome P450 Enzymes. Cytochrome P450: Structure, Mechanism, and Biochemistry*, 3rd ed.; Ortiz de Montellano, P. R., Ed.; Kluwer Academic/Plenum Publishers: New York, 2005. (b) Guengerich, F. P. *Curr. Drug Metab.* **2001**, *2*, 93−115.

(2) (a) Bernadou, J.; Meunier, B. *Adv. Synth. Catal.* **2004**, *346*, 171−184. (b) Chen, M. S.; White, M. C. *Science* **2010**, *327*, 566−571 and references therein. (c) Costas, M.; Mehn, M. P.; Jensen, M. P.; Que, L., Jr. *Chem. Rev.* **2004**, *104*, 939−986.

(3) (a) Slavik, R.; Peters, J.-U.; Giger, R.; Bürkler, M.; Bald, E. *Tetrahedron Lett.* **2010**, *52*, 749−752. (b) Brodie, B. B.; Axelrod, J.; Shore, P. A.; Udenfriend, S. *J. Biol. Chem.* **1954**, *208*, 731−739. *J. Biol. Chem.* **1954**, *208*, 741−750.

(4) (a) Bright, T. V.; Dalton, F.; Elder, V. L.; Murphy, C. D.; O'Connora, N. K.; Sandford, G. *Org. Biomol. Chem.* **2013**, *11*, 1135−1142. (b) St. Jean, D. J., Jr.; Fotsch, C. *J. Med. Chem.* **2012**, *55*, 6002−6020. (c) Park, B. K.; Boobis, A.; Clarke, S.; Goldring, C. E. P.; Jones, D.; Kenna, J. G.; Lambert, C.; Laverty, H. G.; Naisbitt, D. J.; Nelson, S.; Nicoll-Griffith, D. A.; Obach, R. S.; Routledge, P.; Smith, D. A.; Tweedie, D. J.; Vermeulen, N.; Williams, D. P.; Wilson, I. D.; Baillie, T. A. *Nat. Rev. Drug Discov.* **2011**, *10*, 292−306. (d) Kalgutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. *Curr. Drug Metab.* **2005**, *6*, 161−225.

(5) (a) Schroer, K.; Kittelmann, M.; Lütz, S. *Biotechnol. Bioeng.* **2010**, *106*, 699−706. (b) Guengerich, F. P. *Nat. Rev. Drug Discov.* **2002**, *1*, 359−366.
(6) CYPs are responsible for most oxidative mechanisms in drug clearance (including for amines): (a) Gorrod, J. W.; Aislaitner, G. *Eur. J. Drug Metab. Pharmacokinet.* **1994**, *19*, 209−217. (b) Tozer, T. N.; Rowland, M. *Introduction to pharmacokinetics and pharmacodynamics: the quantitative basis of drug therapy*; Lippincott Williams & Wilkins Publishers: Philadelphia, PA, 2006.
(7) MetaSite is a computational tool that predicts the site of metabolism and the isoform selectivity for the main human cytochromes, see: (a) Cruciani, G.; Carosati, E.; De Boeck, B.; Ethirajulu, K.; Mackie, C.; Howe, T.; Vianello, R. *J. Med. Chem.* **2005**, *48*, 6970−6979. (b) Trunzer, M.; Faller, B.; Zimmerlin, B. *J. Med. Chem.* **2009**, *52*, 329−335.
(8) Godula, K.; Sames, D. *Science* **2006**, *312*, 67−72.
(9) Miller-Moslin, K.; Peukert, S.; Jain, R. K.; McEwan, M. A.; Karki, R.; Llamas, L.; Yusuff, N.; He, F.; Li, Y.; Sun, Y.; Dai, M.; Perez, L.; Michael, W.; Sheng, T.; Lei, H.; Zhang, R.; Williams, J.; Bourret, A.; Ramamurthy, A.; Yuan, J.; Guo, R.; Matsumoto, M.; Vattay, A.; Maniara, W.; Amaral, A.; Dorsch, M.; Kelleher, J. F. *J. Med. Chem.* **2009**, *52*, 3954−3968.
(10) (a) Carril, M.; SanMartin, R.; Domínguez, E. *Curr. Org. Chem.* **2007**, *11*, 1385−1399. (b) Grant, S. M.; Faulds, D. *Drugs* **1992**, *43*, 873−888.
(11) Vree, T. B.; Den Biggelaar-Martea, M. V.; Vervey Van Wissen, C. P.; Vree, M. L.; Guelen, P. J. *Br. J. Clin. Pharmacol.* **1993**, *35*, 467−472.
(12) (a) For a review on Cu-mediated C-H activation using molecular oxygen, see: Wendlandt, A. E.; Suess, A. M.; Stahl, S. S. *Angew. Chem., Int. Ed.* **2011**, *50*, 11062−11087. (b) Hoover, J. M.; Stahl, S. S. *J. Am. Chem. Soc.* **2011**, *133*, 16901−16910.
(13) Drug Approvals and Databases, U.S. Food and Drug Administration, http://www.fda.gov/Drugs/InformationOnDrugs.
(14) Löber, S.; Hübner, H.; Tschammer, N.; Gmeiner, P. *Trends Pharmacol. Sci.* **2011**, *32*, 148−157.
(15) (a) Sun, H.; Scott, D. O. *ACS Med. Chem. Lett.* **2011**, *2*, 638−643. (b) Caccia, S. *Curr. Drug Metab.* **2007**, *8*, 612−622.
(16) Intramolecular oxidation of a copper(II)-hydroperoxo dimethylamine ligand into its corresponding formamide was previously reported, see: Maiti, D.; Narducci Sarjeant, A. A.; Karlin, K. D. *J. Am. Chem. Soc.* **2007**, *129*, 6720−6721.
(17) For benzylic oxidations of 1,2,3,4-tetrahydroisoquinolines, see: (a) Lee, N. H.; Lee, C.-S.; Jung, D.-S. *Tetrahedron Lett.* **1998**, *39*, 1385−1388. (b) M.-H. Liu, Y.; Ho, C.-M.; Che, C.-M. *Chem. Asian J.* **2009**, *4*, 1551−1561. For Ru-mediated oxidation of norbornane derivatives or cyclic benzylamines using excess of $NaIO_4$ as oxidant, see: (c) Memeo, M. G.; Mantione, D. P.; Bovio, B.; Quadrelli, P. *Synthesis* **2011**, *13*, 2165−2174. (d) Bettoni, G.; Carbonara, G.; Franchini, C.; Tortorella, V. *Tetrahedron* **1981**, *37*, 4159−4164. For Os-mediated oxidation of tertiary amines, see: (e) Fujii, H.; Ogawa, R.; Jinbo, E.; Tsumura, S.; Nemoto, T.; Nagase, H. *Synlett* **2009**, *14*, 2341−2345. For the oxidation of benzo-fused cyclic amines using nanosized Au clusters stabilized by hydrophilic polymers, see: (f) Preedasuriyachai, P.; Chavasiri, W.; Sakurai, H. *Synlett* **2011**, *8*, 1121−1124. For the oxidation of amides such as 3,4-dihydroisoquinolin-a(2H)-ones and *N*-Boc protected amines, see: (g) Yoshifuji, S.; Arakawa, Y. *Chem. Pharm. Bull.* **1989**, *37*, 3380−3381. (h) Tanaka, K. I.; Yoshifuji, S.; Nitta, Y. *Chem. Pharm. Bull.* **1988**, *36*, 3125−3129. For other specialized examples of stockiometric systems, see: (i) Using Hg: Möhrle, H.; Azodi, K. *Pharmazie* **2006**, *61*, 815−822. (j) Using $KMnO_4$: Markgraf, J. H.; Sangani, P. K.; Finkelstein, M. *Synth. Commun.* **1997**, *27*, 1285−1290.
(18) (a) The S9 fractions used in our study were from 10 different animal sources: rabit, rat, horse, fowl, dog, bovine, pig, sheep, mouse, and chicken; the 14 enzymes were obtained through a collaboration between the Biomedical Research Centre of the University of Dundee and nine pharmaceutical companies (LINK I consortium): (b) Blake, J. A. R.; Pritchard, M. P.; Ding, S.; Smith, G. C. M.; Burchell, B.; Wolf, C. R.; Friedberg, T. *FEBS Lett.* **1996**, *397*, 210−214. (c) Pritchard, M. P.; Glancey, M. J.; Blake, J. A. R.; Gilham, D. E.; Burchell, B.; Wolf, C. R.; Friedberg, T. *Pharmacogenetics* **1998**, *8*, 33−42. (d) Pritchard, M. P.; Ossetian, R.; Li, D. N.; Henderson, C. J.; Burchell, B.; Wolf, C. R.; Friedberg, T. *Arch. Biochem. Biophys.* **1997**, *345*, 342−354. (e) A minimal threshold of 5−10% product formation is needed in the analytical screen in order to proceed to a preparative scale.
(19) The high-dilution volumes typical in biotransformations render scale up unpractical above a certain amount of starting material.
(20) For further details on the chemical conditions investigated, see the SI: (a) For a review on chemical C-H bond oxidations, see: Newhouse, T.; Baran, P. S. *Angew. Chem., Int. Ed.* **2011**, *50*, 3362−3374. (b) Chen, X.; Hao, X.-S.; Goodhue, C. E.; Yu, J.-Q. *J. Am. Chem. Soc.* **2006**, *128*, 6790−6791. (c) Qiu, D.; Zheng, Z.; Mo, F.; Xiao, Q.; Tian, Y.; Zhang, Y.; Wang, J. *Org. Lett.* **2011**, *13*, 4988−4991. (d) Mkhalid, I. A. I.; Barnard, J. H.; Marder, T. B.; Murphy, J. M.; Hartwig, J. F. *Chem. Rev.* **2010**, *110*, 890−931. (e) Chotana, G. A.; Rak, M. A.; Smith, M. R., III. *J. Am. Chem. Soc.* **2005**, *127*, 10539−10544. (f) Fatiadi, A. J. *Synthesis* **1987**, *2*, 85−127. (g) Zhang, Y.-H.; Yu, J.-Q. *J. Am. Chem. Soc.* **2009**, *131*, 14654−14655. (h) Dick, A. R.; Hull, K. L.; Sanford, M. S. *J. Am. Chem. Soc.* **2004**, *126*, 2300−2301. (i) Zhang, S.; Luo, F.; Wang, W.; Jia, X.; Hu, M.; Cheng, J. *Tetrahedron Lett.* **2010**, *51*, 3317−3319. (j) Dangel, B. D.; Johnson, J. A.; Sames, D. *J. Am. Chem. Soc.* **2001**, *123*, 8149−8150. (k) McNeill, E.; Du Bois, J. *J. Am. Chem. Soc.* **2010**, *132*, 10202−10204. (l) Murray, R. W.; Iyanar, K.; Chen, J.; Wearing, J. T. *Tetrahedron Lett.* **1995**, *36*, 6415−6418. (m) O'Malley, D. P.; Yamaguchi, J.; Young, I. S.; Seiple, I. B.; Baran, P. S. *Angew. Chem., Int. Ed.* **2008**, *47*, 3581−3583. (n) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229−246.
(21) (a) For recent exploratory Cu-mediated benzylic oxidations, see: Würtele, C.; Sander, O.; Lutz, V.; Waitz, T.; Tuczek, F.; Schindler, S. *J. Am. Chem. Soc.* **2009**, *131*, 7544−7545. (b) Lucas, H. R.; Li, L.; Narducci Sarjeant, A. A.; Vance, M. A.; Solomon, E. I.; Karlin, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 3230−3245. (c) De Houwer, J.; Tehrani, K. A.; Maes, B. U. W. *Angew. Chem., Int. Ed.* **2012**, *51*, 2745−2748.
(22) The Cu-mediated benzylic oxidation had not been examined on systems related to **1** and complex drugs.
(23) The cleavage of formamides to afford *N*-dealkyted amines is well precedented under acidic conditions and mild heating: Vachal, P.; Jacobsen, E. N. *Org. Lett.* **2000**, *2*, 867−870.
(24) Gschwind, H.-P.; Pfaar, U.; Waldmeier, F.; Zollinger, M.; Sayer, C.; Zbinden, P.; Hayes, M.; Pokorny, R.; Seiberling, M.; Ben-Am, M.; Peng, B.; Gross, G. *Drug Metabol. & Disp.* **2005**, *33*, 1503−1512.
(25) Soledade, M.; Pedras, S.; Yu, Y. *Bioorg. Med. Chem.* **2008**, *16*, 8063−8071.
(26) For a review on the synthetic access to oxidized-piperazine derivatives, see: Dinsmore, C. J.; Beshore, D. C. *Tetrahedron* **2002**, *58*, 3297−3312.
(27) Witiak, D. T.; Wei, Y. *Prog. Drug. Res.* **1990**, *35*, 249−263.
(28) Pagano, T. G.; Gong, Y.; Kong, F.; Tsao, R.; Fawzi, M.; Zhu, T. *J. Antibiot.* **2011**, *64*, 673−677.
(29) Hu, Y.; Wang, K.; MacMillan, J. B. *Org. Lett.* **2013**, *15*, 390−393.