Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 2975–2980
Bioorganic & Medicinal Chemistry

Studies on the cytochrome P450 catalyzed oxidation of ^{13}C labeled 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine by ^{13}C NMR

Philippe Bissel and Neal Castagnoli, Jr.*

Department of Chemistry, Virginia Tech, Blacksburg, VA 24061-0212, USA

Received 21 December 2004; accepted 1 February 2005

Abstract—A recent study from Hanzlik's laboratory (*J. Am. Chem. Soc*. **2002**, *124*, 8268) has provided compelling evidence of a hydrogen atom transfer pathway for the cytochrome P450-catalyzed oxidative N-decyclopropylation of *N*-cyclopropyl-*N*-methylaniline. In the present paper, we report an analogous pathway for the oxidative decyclopropylation of a ^{13}C-labeled 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridinyl substrate. Three ^{13}C-enriched metabolites were characterized: (1) a diastereomeric pair of *N*-cyclopropyl-*N*-oxides; (2) the *N*-cyclopropylpyridinium species; and (3) cyclopropanone hydrate. These results extend the hydrogen atom transfer pathway to include aliphatic tertiary amine substrates. Consideration of all of the available evidence, however, leads us to conclude that the cytochrome P450-catalyzed α-carbon oxidations of cyclopropylamines may proceed via both the single electron and hydrogen atom transfer pathways.

1. Introduction

The cytochrome P450s (cP450s) constitute a family of monoxygenases that catalyze a variety of oxidative biotransformations[1] including the α-carbon oxidation of tertiary amines (**1**). As illustrated in Scheme 1, the iron oxo system [Fe^{3+}(O)] is the electron acceptor for the overall 2-electron oxidation leading to the iminium metabolite **5**.[2] One pathway often invoked to account for this biotransformation involves an initial single electron transfer (SET) from the nitrogen lone pair[3] to form the aminyl radical cation **2** followed by deprotonation to give the carbon radical intermediate **3**. An alternative pathway proceeds by an initial hydrogen atom transfer (HAT)[4] to generate the same radical intermediate **3** without passing through the aminyl radical cation. The second 1-electron oxidation to form the iminium product **5** is thought to proceed via a radical recombination process that gives the α-hydroxy intermediate **4** (Scheme 1).

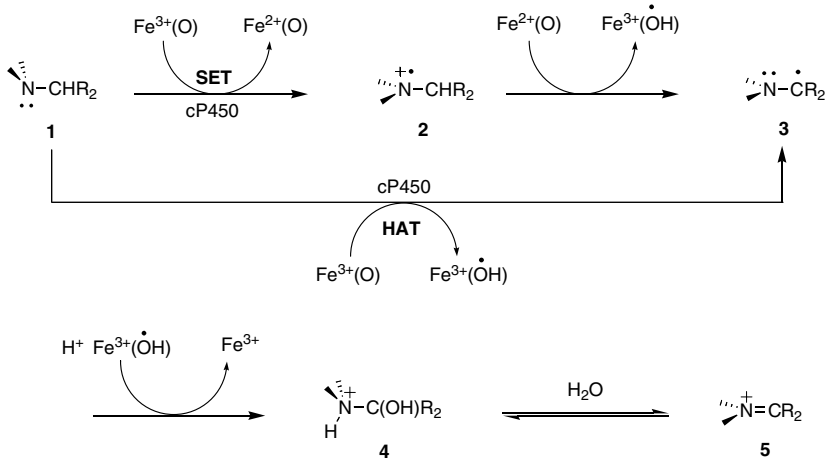
The cP450 mechanism-based inactivator properties of *N*-cyclopropylbenzylamine (**6**)[5] are consistent with a SET pathway. According to this proposal, the cyclopropyl ring of the SET generated aminyl radical cation **7** opens, via the bisected conformation shown in Scheme 2, to give the distonic primary carbon radical cation **8** that covalently modifies the active site of the enzyme via the adduct **9**. Critical supporting evidence for this proposal is the observation that the corresponding α-methyl analog **10**, which cannot undergo an HAT reaction, still inactivates the enzyme. This is consistent with the reaction sequence **10** → **11** → **12** → **13** (Scheme 2).

Also noteworthy are recent ab initio calculations suggesting that the bisected conformation **15** of the aminyl radical cation derived from cyclopropylamine **14** does not exist as a discrete species.[6] Instead, the SET pathway leads directly to the distonic radical cation **16** (Scheme 3). Related computational studies on cyclopropylaminyl radical cations in general support this analysis.[7]

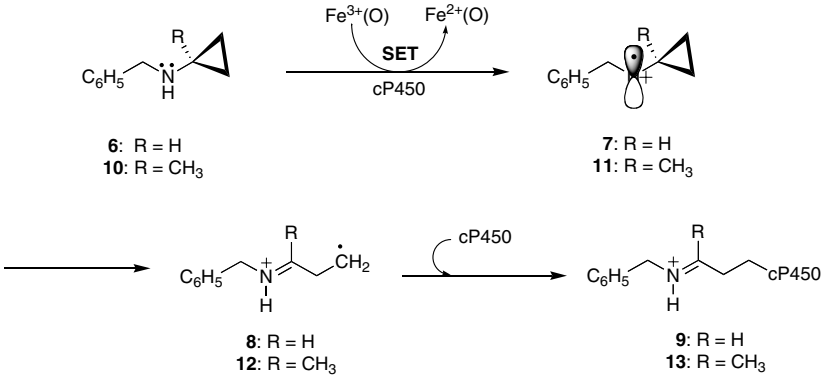
Indirect evidence supporting an HAT pathway includes the results of studies on the cP450 catalyzed oxidative metabolism of *N*-cyclopropyl-*p*-chloroanilines bearing *N*-methyl and *N*-isopropyl groups.[8] When the α-proton at the C-1 position of the cyclopropyl group was replaced by a methyl group, metabolite formation shifted from a mixture of N-dealkylated and N-decyclopropylated metabolites to N-dealkylated products only. A

* Corresponding author. Fax: +1 540 231 8890; e-mail: ncastagn@vt.edu

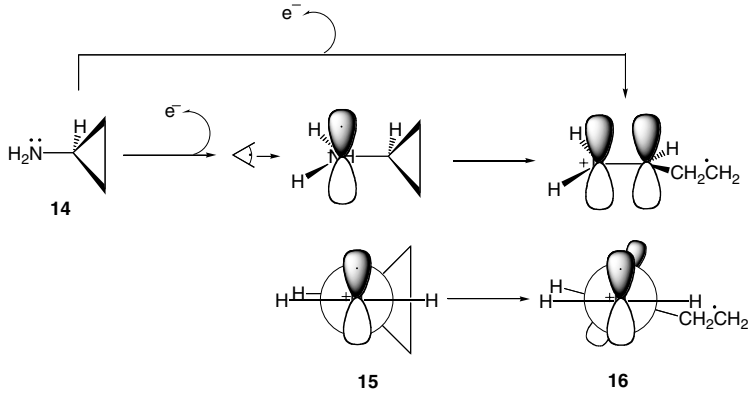
0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.02.004



Scheme 1.



Scheme 2.



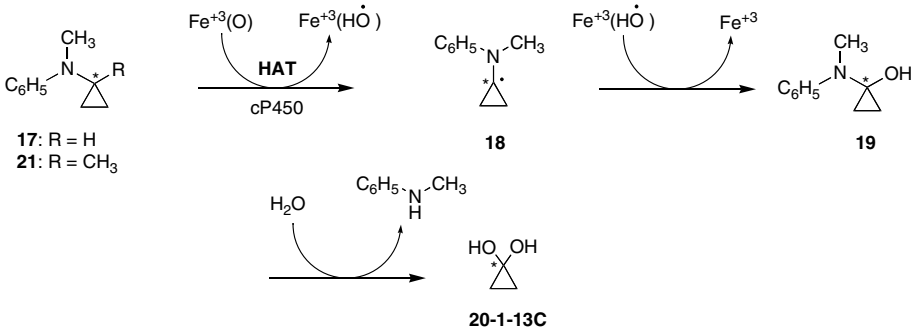
Scheme 3.

deuterium isotope effect favoring N-dealkylation of about 3 was also observed when this C-1 proton was replaced by a deuteron. Finally, only N-decyclopropylation, presumably via a ring opening pathway, was observed in a model reaction employing the one-electron oxidant $[Fe^{3+}(Phen)_3(PF_6^-)_3]$.

Direct evidence for the HAT pathway is the characterization of the ^{13}C-labeled cyclopropanone hydrate **20-1-^{13}C** as a cP450 generated metabolite of the ^{13}C-labeled *N*-cyclopropyl-*N*-methylaniline substrate **17**.[9] The corresponding α-methyl analog **21** did not undergo the decyclopropylation reaction. The authors proposed the HAT pathway (**17** → **18** → **19** → **20**) shown in Scheme 4 to accommodate these results.

2. Results and discussion

We have been interested in the metabolic fate of 1,4-disubstituted 1,2,3,6-tetrahydropyridines in part because the parkinsonian inducing properties of the selective

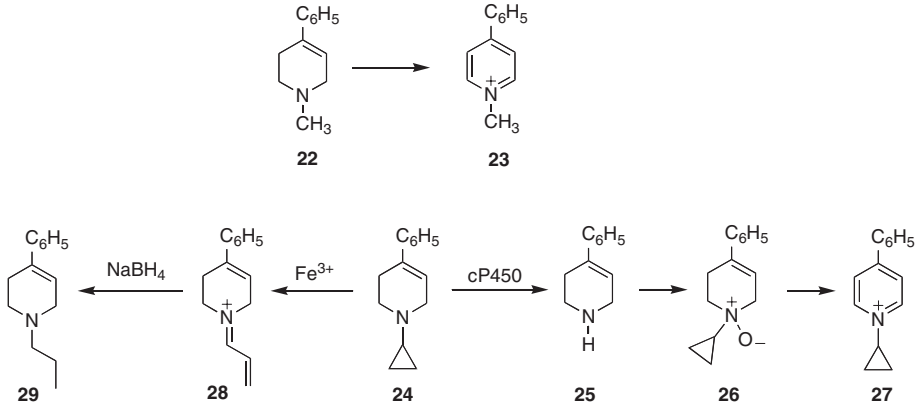


Scheme 4.

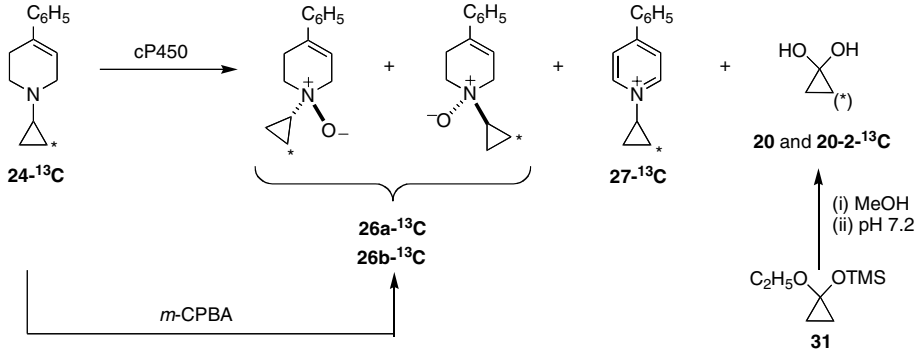
nigrostriatal neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**22**)] are mediated by its pyridinium metabolite **23**.[10] As part of these studies we observed that the rat liver microsomal enzyme catalyzed oxidation of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine (**24**) led to a mixture of products that included the secondary amine **25**, the N-oxide **26** and the pyridinium species **27** (Scheme 5).[11] Attempts to characterize the fate of the cyclopropyl group was not successful. In contrast to the enzyme mediated products, chemical model studies established that the 1-electron oxidant $[Fe^{3+}(Phen)_3(PF_6^-)_3]$ oxidized **24** to yield only ring-opened products including the eniminium species **28** that was 'trapped' by reduction with $NaBH_4$ to give the *N*-propyl analog **29**.[12]

In an attempt to gain further insight in the mechanism leading to the cP450-mediated decyclopropylation of **24**, we have examined the metabolic fate of the corresponding ^{13}C-labeled analog **24-^{13}C** using rat liver microsomes and an NADPH generating system (Scheme 6). A parallel incubation was carried out under identical conditions except that no substrate was present (see Experimental). The ^{13}C NMR spectrum of the incubation mixture containing the substrate shown in Figure 1 was prepared by subtracting all background signals observed in an identical incubation not containing the substrate.

The spectrum shows five signals. The lowest field signal at δ 17.5 ppm is likely to be a minor ^{13}C-enriched



Scheme 5.



Scheme 6.

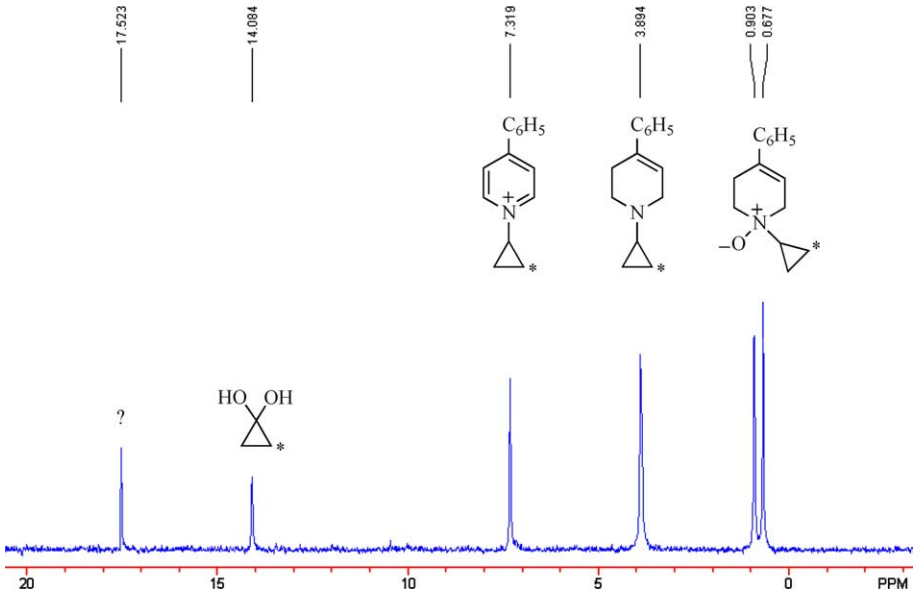
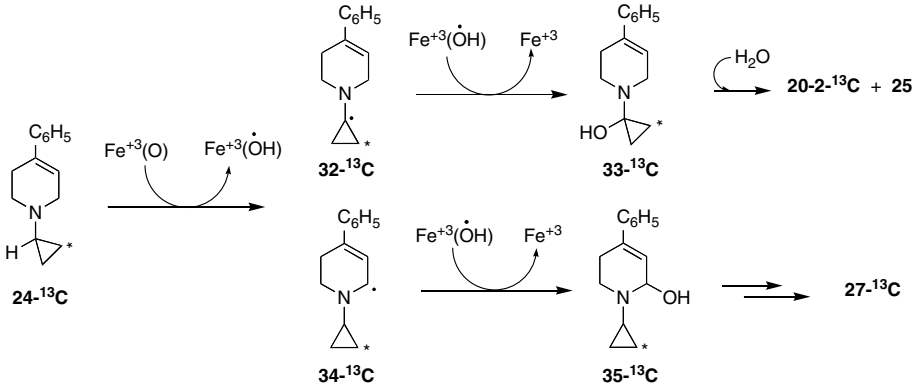


Figure 1. ^{13}C NMR spectrum of the incubation mixture with the substrate **24-^{13}C**.

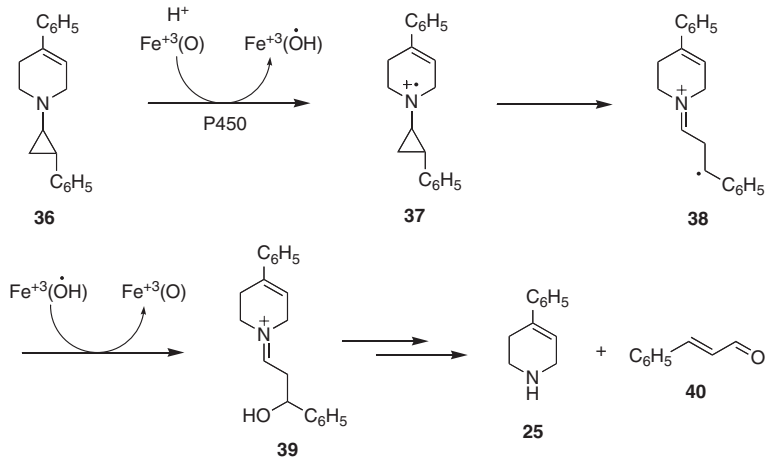
impurity since it was present as a weak signal in the spectrum of the starting material and could not be assigned. The signal at δ 14.1 ppm was assigned to cyclopropanone-2-^{13}C hydrate (**20-2-^{13}C**). This assignment was confirmed by the ^{13}C spectrum of an authentic standard of unlabeled cyclopropanone hydrate (**20**) that was prepared by treating commercially available [(1-ethoxycyclopropyl)oxy]trimethylsilane (**31**) first with methanol and then with aqueous buffer (pH 7.2) (Scheme 6). The signal at δ 7.3 ppm could be assigned to the pyridinium species **27-^{13}C** by comparison with the spectrum of unlabeled synthetic **27**. The signal at 3.9 ppm was due to the substrate **24-^{13}C**. The two equally intense signals at 0.9 and 0.7 ppm were assigned to the diastereomeric N-oxides **26a-^{13}C** and **26b-^{13}C**. The diastereoisomerism observed with these N-oxides is a consequence of the presence of the nitrogen and the C-1 cyclopropyl stereogenic centers, the chirality of the latter center being a consequence of the presence of the ^{13}C-enriched atom. A synthetic standard of **26a-^{13}C** and **26b-^{13}C**, prepared by the oxidation of **24-^{13}C** with *m*-CPBA, confirmed these assignments.

The results of these studies extend to an sp^3-hybridized aminyl system evidence that the cP450s catalyze the oxidative decyclopropylation of cyclopropylamines via an HAT pathway. The conversion of **24-^{13}C** to **20-2-^{13}C** by NADPH supplemented rat liver microsomal preparations is proposed to proceed by an initial HAT from the substrate to give the cyclopropylmethide radical **32-^{13}C** (Scheme 7). Radical recombination with the heme-stabilized hydroxyl radical generates the α-carbinolamine **33-^{13}C** that will cleave to give, after addition of water, ^{13}C-labeled cyclopropanone hydrate (**20-2-^{13}C**) analogous to the pathway demonstrated by Hanzlik et al. for the aniline systems **17** and **21**.[9] The corresponding pathway (**24-^{13}C** → **34-^{13}C35-^{13}C** → **27-^{13}C**) may account for the formation of the pyridinium metabolite **27-^{13}C**.

Although the evidence presented here and in the cited literature, support an HAT pathway for the cP450-catalyzed α-carbon oxidation of tertiary amines, results from an earlier study on the cP450-catalyzed oxidation of 4-phenyl-1-(*trans*-2-phenyl)cyclopropylamine **36** are best



Scheme 7.



Scheme 8.

rationalized by a SET pathway (Scheme 8).[13] In this case, at least part of the phenylcyclopropyl moiety of **36** is converted to cinnamaldehyde (**40**). An obvious route to cinnamaldehyde proceeds via SET to give the cyclopropylaminyl radical cation **37** that ring opens to the distonic radical cation **38**. Radical recombination, followed by cleavage and dehydration of intermediate **39**, yields secondary amine **25** and cinnamaldehyde (**40**). The stability of the benzylic radical present in **38** may contribute to the preference of the SET pathway observed in this biotransformation.

Consideration of all of the available evidence leads us to conclude that the cP450s catalyzed α-carbon oxidation of cyclopropylamines and, perhaps, of tertiary amines in general, may proceed by both the HAT and SET pathway. It should be kept in mind that ring opening of a cyclopropylaminyl radical cation will proceed via the bisected conformation and therefore conformational restrictions imposed by the active site could impact on the fate of these species. Studies are being pursued to identify what factors contribute to the dominance of one pathway over the other.

3. Experimental

The THF was distilled from sodium and benzophenone. Proton and ^{13}C NMR spectra were recorded on a JEOL 500-MHz spectrometer. Compounds **20**,[9] **24-^{13}C**,[14] and **27**[11] were synthesized according to the literature. The high resolution FAB mass spectrum was obtained on a JEOL HX-110 instrument. Male rat liver microsomes (20 mg protein/mL) were purchased from XenoTechLLC. The substrate in the absence of the enzyme was shown by ^{13}C NMR to be stable for 48 h at 37 °C. Incubations (10 mL in 0.1 M phosphate buffer, pH 7.4) were performed in capped Erlenmeyer flasks at 37 °C for 48 h. The sample incubation mixture contained $MgCl_2$ (4.0 mM), substrate **24-^{13}C** (0.5 mM), microsomal protein (2.0 mg), β-$NADP^+$ sodium salt (0.4 mM), D,L-isocitrate trisodium salt (2.0 mM), and isocitrate dehydrogenase (2 units). A control incubation (no substrate) was performed under identical conditions. At the end of the incubations, the mixtures were treated with acetonitrile (10 mL) and the resulting mixtures were centrifuged at 10,000*g* for 10 min. The supernatants were concentrated under reduced pressure and D_2O (1 mL) was added to the residues. The solutions were filtrated before obtaining the 500 MHz NMR spectra (NA = 69,000).

3.1. 1-(2-^{13}C-Cyclopropyl)-4-phenyl-1,2,3,6-tetrahydropyridine-*N*-oxides (26a-^{13}C and 26b-^{13}C)[15]

To a stirred solution of the free base **24-^{13}C** (68 mg, 0.34 mmol) in CH_2Cl_2 (10 mL) was added 67% *m*-CPBA (124 mg, 0.48 mmol) at 0 °C. After 15 min at room temperature, the solvent was removed under reduced pressure and the crude product was purified by chromatography on neutral alumina to yield a 1 to 1 mixture of diastereomers **26a-^{13}C** and **26b-^{13}C** as a hygroscopic, white solid (62 mg, 84%): ^1H NMR (500 MHz, $CDCl_3$) δ 0.59 (m, 2H), 1.50 (m, 2H), 2.79 (m, 1H), 3.09, (m, 2H), 3.58 (m, 2H), 4.06 (m, 1H), 4.18 (m, 1H), 5.98 (m, 1H), 7.36 (m, 5H); ^{13}C NMR (125.8 MHz, D_2O) δ 0.61, 0.87, 24.3, 48.1, 48.3, 62.5, 66.1, 116.4, 125.2, 128.3, 129.0, 134.2, 138.7; FAB-HRMS: Calcd for $^{12}C_{13}{}^{13}CH_{18}NO^+$: 217.1422. Found: 217.1427. Anal. Calcd for $^{12}C_{13}{}^{13}CH_{17}NO{\cdot}0.48H_2O$ (224.93): C, 75.17; H, 8.04; N, 6.23. Found: C, 75.17; H, 8.02; N, 6.23.

3.2. Cyclopropanone hydrate (20)[9]

This compound, prepared by the literature procedure, was obtained in 88% yield. The ^{13}C NMR spectrum was essentially identical to that reported previously. Reported:[9] ^{13}C NMR (125.8 MHz, D_2O) δ 13.8, 79.4. This study: (125.8 MHz, D_2O) δ 14.0, 79.7.

Acknowledgements

This work was supported by the Harvey W. Peters Center for the Study of Parkinson's Disease.

References and notes

1. Guengerich, P. F. *Chem. Res. Toxicol.* **2001**, *14*, 611.
2. Hlavick, P. *Drug Metab. Rev.* **2002**, *34*, 451; Karki, S. B.; Dinnocenzo, J. P. *Xenobiotica* **1995**, *25*, 711; Rose, J.; Castagnoli, N., Jr. *Med. Res. Rev.* **1983**, *3*, 73; Silverman, R. B.; Hoffman, S. J.; Catus, W. B. *J. Am. Chem. Soc.* **1980**, *102*, 7126.
3. Sato, H.; Guengerich, F. P. *J. Am. Chem. Soc.* **2000**, *122*, 8099; Guengerich, F. P.; Yun, C. H.; Macdonald, T. L. *J. Biol. Chem.* **1996**, *271*, 27321; Guengerich, F. P.; Bell, L. C.; Okazaki, O. *Biochimie* **1995**, *77*, 573; Guengerich, F. P.; Okazaki, O.; Seto, Y.; Macdonald, T. L. *Xenobiotica* **1995**, 689; Bondon, A.; Macdonald, J. L.; Harris, T. M.; Guengerich, F. P. *J. Biol. Chem.* **1989**, *264*, 1988; Mcdonald, T. L. *J. Am. Chem. Soc.* **1984**, *106*, 6446; Augusto, O.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 11288.
4. Manchester, J. I.; Dinnocenzo, J. P.; Higgins, L. A.; Jones, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 5069; Karki, S. G.; Dinnocenzo, J. P.; Jones, J. P.; Korzekwas, K. R. *J. Am. Chem. Soc.* **1995**, *117*, 2732; Dinnocenzo, J. P.; Karki, S. B.; Jones, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 7111.
5. Hanzlik, R. P. *J. Med. Chem.* **1979**, *22*, 759; Macdonald, T. L.; Zirvi, K.; Burka, L. T.; Peyman, P.; Guengerich, F. P. *J. Am. Chem. Soc.* **1982**, *104*, 2050; Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048.
6. Nguyen, T. M.; Creve, S.; Ha, T. K. *Chem. Phys. Lett.* **1998**, *293*, 90.
7. Coosky, L. A.; King, H. F.; Richardson, W. H. *J. Org. Chem.* **2003**, *68*, 9441.
8. Bhakta, N. M.; Wimalasena, K. *J. Am. Chem. Soc.* **2002**, *124*, 1844.
9. Shaffer, C. L.; Harriman, S.; Koen, Y. M.; Hanzlik, R. P. *J. Am. Chem. Soc.* **2002**, *124*, 8268.
10. Chiba, K.; Trevor, A. J.; Castagnoli, N., Jr. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 574; Youdim, M. B. H. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1989**, *13*, 363; Glover, V.; Gibb, C.; Sandler, M. *Neurosci. Lett.* **1986**, *64*, 216; Cohen, G. *Ann. N.Y. Acad. Sci.* **2000**, *899*, 112.
11. Zhao, Z.; Mabic, S.; Kuttab, S.; Franot, C.; Castagnoli, K., Jr.; Castagnoli, N., Jr. *Bioorg. Med. Chem.* **1998**, *6*, 2531.
12. Franot, C.; Mabic, S.; Castagnoli, N., Jr. *Bioorg. Med. Chem.* **1998**, *6*, 283.
13. Kuttab, S.; Shang, J.; Castagnoli, N., Jr. *Bioorg. Med. Chem.* **2001**, *9*, 1685.
14. Kuttab, S.; Mabic, S. *J. Label. Compd. Radiopharm.* **2002**, *45*, 813.
15. Hall, L.; Murray, S.; Castagnoli, K.; Castagnoli, N., Jr. *Chem. Res. Toxicol.* **1992**, *5*, 625.