Metalloporphyrins as chemical mimics of cytochrome P-450 systems
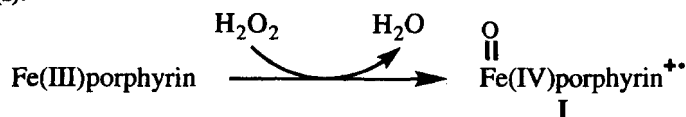
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Abstract: Certain synthetic metalloporphyrins have been shown to mimic the *in vivo* metabolism of some pharmaceuticals. Oxidation, hydroxylation and N-demethylation yielded synthetic metabolites. If found to be general, this lays the foundation of a predictive basis to optimize analog design of inhibitors with reduced oxidative reactivity, to determine the proclivity of drugs to form biologically active metabolites and provides a convenient methodology for their preparation.

Introduction:

In humans and other animals, most drugs are metabolized in the liver. Many drug metabolites are formed by oxidative mechanisms catalyzed primarily by heme and cytochrome containing enzymes.[1] Most biological oxidations involve primary catalysis provided by the cytochrome P-450 mono-oxygenase enzymes. All heme proteins that are activated by hydrogen peroxide, including catalases, peroxidases and ligninases function via a two electron oxidation of the ferric resting state to an oxoferryl porphyrin π-cation radical (I).[2]

$$\text{Fe(III)porphyrin} \xrightarrow{\quad H_2O_2 \quad\quad H_2O \quad} \overset{O}{\underset{I}{\overset{\|}{\text{Fe(IV)porphyrin}}}}^{+\bullet}$$

While this oxidation state has yet to be characterized for the cytochromes P-450, most of their reactions and those of the biomimetic analogs can be accounted for by oxygen transfer from (I) to a variety of substrates to give characteristic reactions such as hydroxylation, epoxidation and heteroatom oxidation.[2] Other products resulting from hydroxyl and hydroperoxyl radicals have also been detected.

The metabolic processes *in vivo* contribute in substantial measure to the efficacy, side effects and also the toxicity of a pharmaceutical entity. These factors are responsible for the success of failure of a clinical candidate. Metabolic processes of drugs are always the subject of intense scrutiny in pharmaceutical companies.

Pharmacologists have traditionally been involved with isolation and identification of the metabolites of a drug. It is imperative to conduct such studies early in the drug development process. Toxicological and pharmacological studies on the metabolites form a crucial segment in the identification of a clinical candidate.

Current problems:

Several problems are currently associated with the use of biological systems in studying drug metabolism:

(i)   *In vitro* studies produce very small quantities of the product. Primary metabolites are often hydrophilic and difficult to isolate.

(ii) Animal studies necessitate the sacrifice of animals and are extremely expensive to conduct. Liver slice preparations are of variable potency; it is difficult to quantitate the precise stoichiometry of the oxidant.

(iii) Pharmacologists do not know, in advance, the structure of the metabolites they should seek.

(iv) Many of the metabolites are not amenable to organic synthesis by conventional routes.

We therefore turned to the metalloporphyrins as mimics of the *in vivo* metabolic processes.

The oxometalloporphyrins:

Synthetic metalloporphyrins have received a lot of recent attention as mimics of numerous enzymes. In addition to models for peroxidases and particularly the ligninases, metalloporphyrins have also found utility as model systems for studies of the oxidative metabolism of drugs.[4-6] A detailed study of the metabolism of lidocaine has been reported[7,] as have preliminary studies on the use of metalloporphyrins as chemical mimics of cytochrome P-450 systems.[8]

The first synthetic metalloporphyrins were found to be oxidatively labile. Few catalytic turnovers were seen due to the rapid destruction of the porphyrin macrocycle. Dolphin showed that introduction of halogens onto the aryl groups (of *meso*-tetraarylporphyrins) and on the ß-pyrrolic positions of the porphyrins increases the turnover of catalytic reactions by decreasing the rate of porphyrin destruction.[9] In addition, the combined electronegativities of the halogen substituents are transmitted to the metal atom making the corresponding oxo-complexes more electron deficient and thus more effective oxidation catalysts.[10]
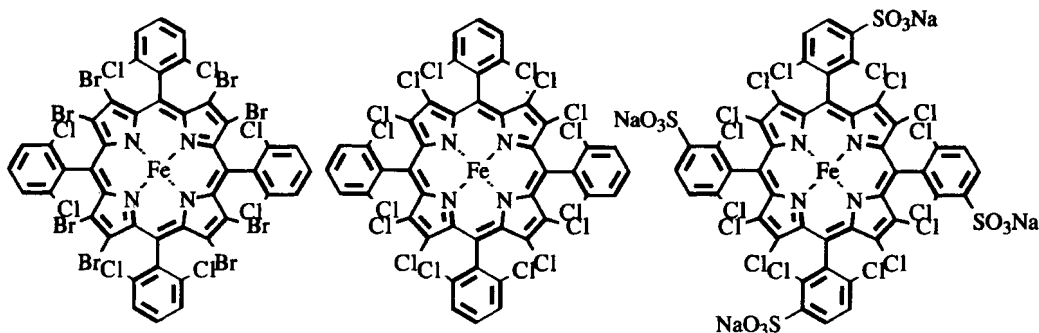

Fig. 1: Metalloporphyrins Used for Our Studies

Synthesis of the Sterically Protected and Electronically Activated Metalloporphyrins:

The past decade has seen considerable advances in the development of synthetic metalloporphyrin complexes.[11-18] We have now designed numerous practical and efficacious methods of synthesizing porphyrins with halogens at the *ortho*-aryl and also the ß-pyrrole positions.[19] The methodology provides facile access to a large number of porphyrins in optimum yields and purity.
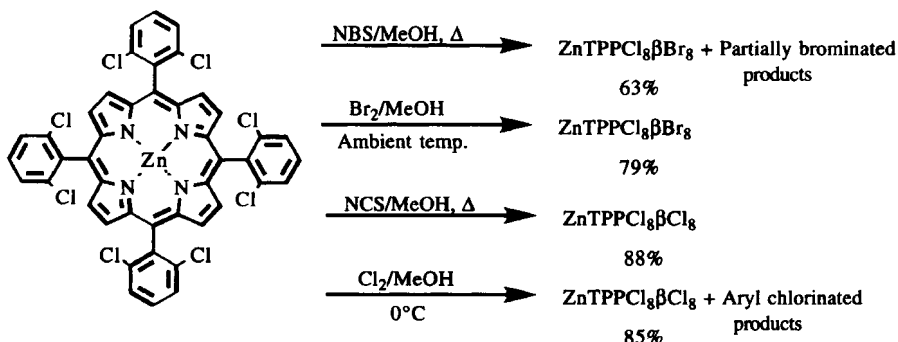

Fig. 2: Improved Methods for the Halogenation of Sterically Hindered Metalloporphyrins

Demetallation with trifluoroacetic acid followed by insertion of iron yielded the corresponding hemins[20].

Application of the Methodology to Selected Drugs:

We have recently applied this technique to the study of the oxidative metabolism of several drugs. Reactions with tetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl, octachloro tetrakis(2,6-dichlorophenyl) porphyrin Fe(III)Cl and octabromo tetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl were conducted in methylene chloride/water or water/acetonitrile (80:20, v/v) at ambient temperatures. Iodosobenzene, cumene hydroperoxide, hydrogen peroxide or sodium hypochlorite were used as the source of exogenous oxygen. Products were separated by HPLC and revealed identity with authentic reference samples of the major metabolites of these drugs previously isolated from the urine of rats or characterized from rat liver microsomal incubations.
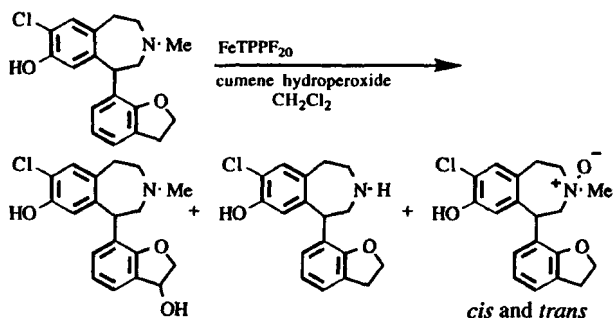
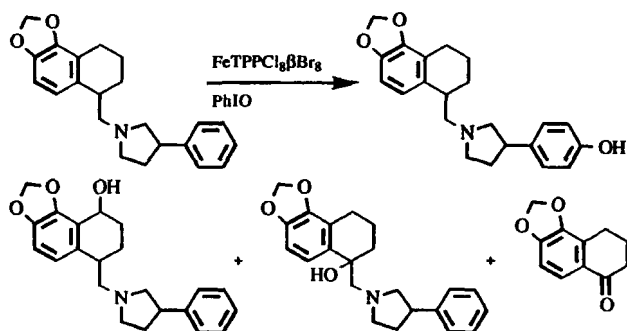**Fig. 3:** Oxidation of Odapipam (Anti-psychosis Drug)

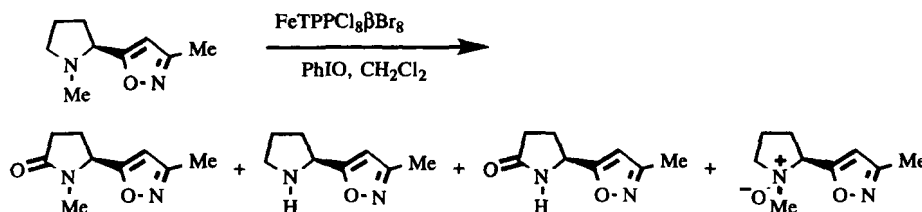**Fig. 4:** Oxidation of ABT-200 (an Anti-depressant Drug)

Fig. 5: Oxidation of ABT-418 (Cholinergic Channel Activator used for the treatment of Alzheimer's Disease)
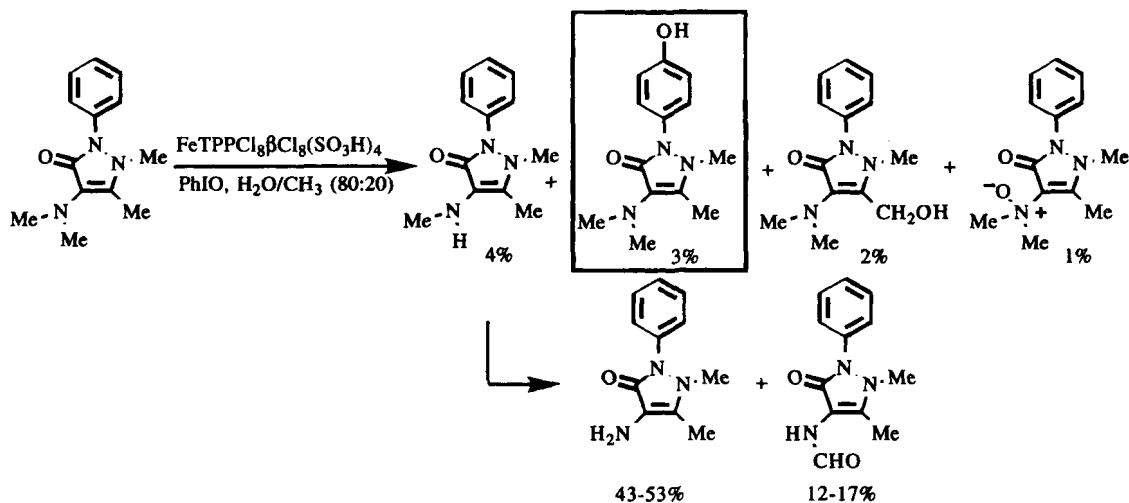
Fig. 6: Oxidation of Aminopyrine

The oxidation of aminopyrene was particularly interesting in that new, hitherto unobserved metabolites were detected. Variation of the reaction conditions then led to the synthesis of selected metabolites in increased yields.
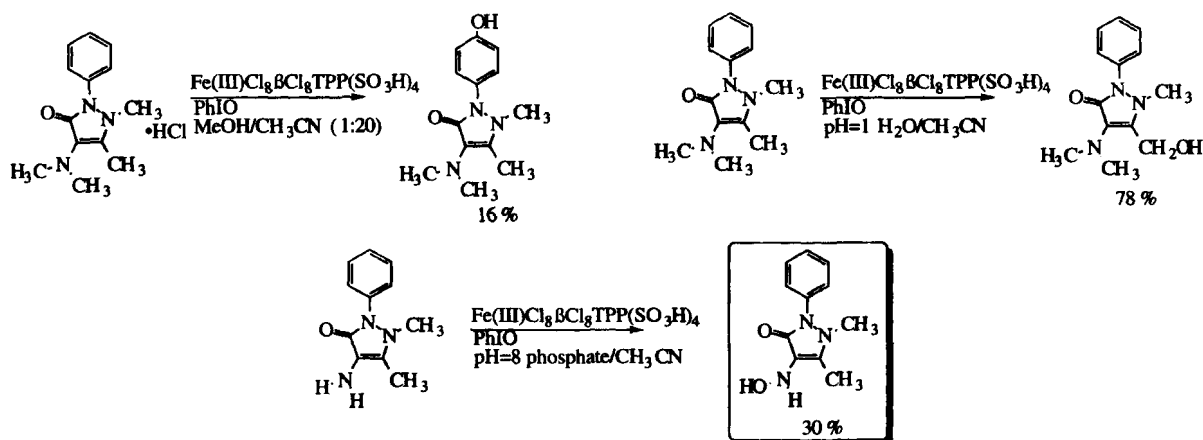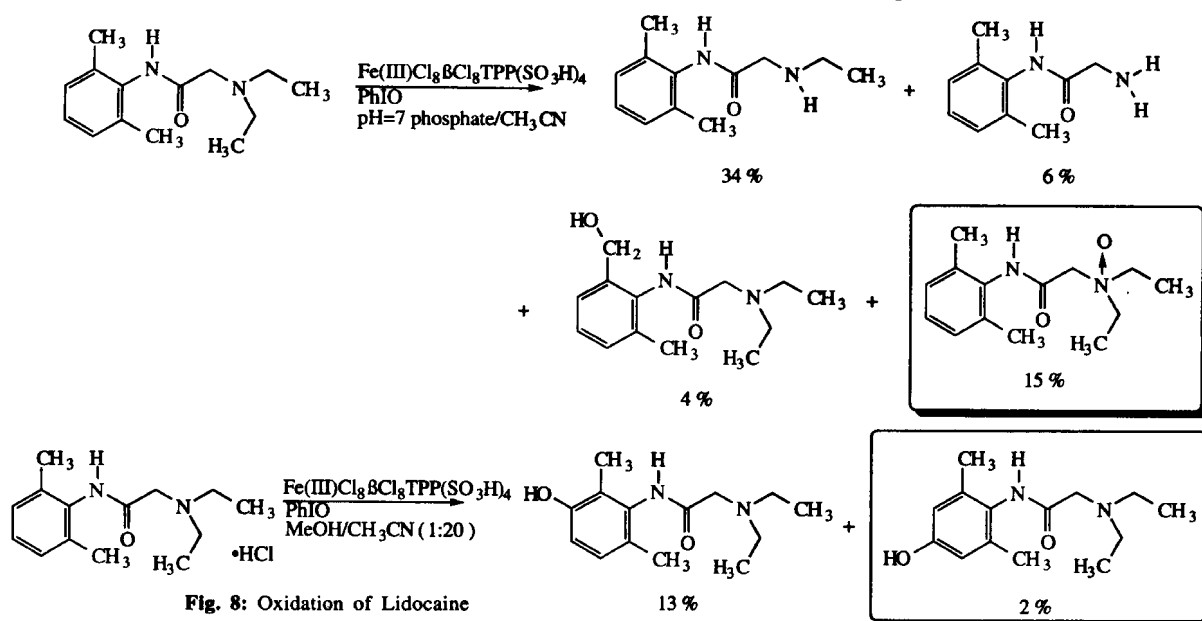
Fig. 7: Selective Oxidation of Aminopyrine

This was also exemplified by the oxidation of lidocaine; two new oxidation products were observed.

Fig. 8: Oxidation of Lidocaine

These had escaped detection in all previous studies.

It is useful to note that these are rare examples of porphyrin mediated oxidation of sophisticated pharmaceutical entities. The reactions described above are generally applicable and have been used in our laboratories to achieve hydroxylation and N-demethylation on numerous other substrates. The hydroxy metabolite and the desmethyl derivative were usually difficult to synthesize by conventional organic chemistry methods.

This approach affords an efficient method for the systematic preparation and identification of the entire spectrum of metabolites from a chosen drug. We can envision the screening of a series of compounds by reacting them with different permutations of a metalloporphyrin, co-oxidant and a suitable solvent. Reaction conditions that produce the maximum number of metabolites can be defined. This logically leads to subsequent scaled-up optimal processes. The oxidation products can then be separated and subjected to toxicologic, pathologic, histopathalogic, or genotoxic testing.

References:
1. P. R. Ortiz de Montellano, in: *Structure, Mechanism and Biochemistry*, p217, P. R. Ortiz de Montellano, Ed., Plenum Press, New York, (1986).
2. D. Dolphin, L. Y. Xie, in: *Metalloporphyrin Catalyzed Oxidations*, p269, F. Montanari and L. Casella, Eds., Kluwer Academic Publishers, The Netherlands, (1994).
3. D. Dolphin, in: *The Activation of Dioxygen and Homogeneous Catalytic Oxidation*, p287, D. H. R. Barton, A. E. Martell, D. T. Sawyer, Eds., 5th International Symposium, College Station Texas, March 14-19, 1993, Plenum Press, New York, (1993).
4. B. Meunier, J. Bernadou, M. Bonnapous, S. Defrance, P. Loiseau, M. Vidal, *Drug Metabolism and Disposition*, **21**, 811, (1993).
5. M. Hirobe, H. Masumoto, S. Ohta, *Drug Metabolism and Disposition*, **19**, 768, (1991).
6. T. Higuchi, M. Hirobe, Y. Nagatsu, *Chem Pharm. Bull.*,**38**, 400, (1990).
7. P. Battioni, M. N. Carrier, D. Mansuy, *Bull. Soc. Chim. Fr.*, **130**, 405, (1993).
8. M. S. Chorghade, D. A. Dezaro, D. R. Hill, E. C. Lee, R. J. Pariza, J. V. Andersen, K. T. Hansen, D. H. Dolphin, *Bioorg. Med. Chem. Lett.*, **4**, 2867, (1994).
9. J. P. Collman, X. Zhang, V. J. Lee, E. S. Uffelman, J. I. Brauman, *Science*, **261**, 1404-1411, (1993).
10. B. Meunier, *Chem. Rev.*, **92**, 1411-1456, (1992).
11. P. Battioni, J. F. Bartoli, D. Mansuy, Y. S. Byun, T. G. Traylor, *J. Chem. Soc., Chem. Commun.*, 1051-1053, (1992).
12. T. G. Traylor, K. W. Hill, W. Fann, S. Tsuchiya, B. E. Dunlop, *J. Am. Chem. Soc.*, **114**, 1308-1312, (1992).
13. P. Hoffmann, A. Robert, B. Meunier, *Bull. Soc. Chim. Fr.*, **129**, 85-97, (1992).
14. T. Nakano, T. G. Traylor, D. Dolphin, *Can. J. Chem.* **68**, 1504-1506, (1990).
15. P. E. Ellis Jr., J. E. Lyons, *Catalysis Lett.*, **3**, 389-398, (1989).
16. J. P. Renaud, P. Battioni, J. F. Bartoli, D. Mansuy, *J. Chem. Soc., Chem. Commun.*, 888-889, (1985).
17. P. S. Traylor, D. Dolphin, T. G. Traylor, *J. Chem. Soc., Chem. Commun.*, 279-280, (1984).
18. J. S. Lindsey, K. A. MacCrum, J. S. Tyhonas, Y.-Y. Chuang, *J. Org. Chem.*, **59**, 579, (1994).
19. M. S. Chorghade, D. Dolphin, D. Dupré, D. R. Hill, E. C. Lee, T. Wijesekera, *Synthesis*, (1995), Submitted for publication.
20. H. Kobayashi, T. Higushi, Y. Kaizu, H. Osada, M. Aoki, *Bull. Chem. Soc. Jpn.*, **48**, 3137-3141, (1975).