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# Oxidative aromatization of 1,4-dihydropyridines and pyrazolines using HbA–$H_2O_2$: An efficient biomimetic catalyst system providing metabolites of drug candidates

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ABSTRACT

Human hemoglobin (HbA) efficiently catalyses the oxidative aromatization of 1,4-dihydropyridines (1,4-DHPs) and pyrazolines with hydrogen peroxide in phosphate buffer. The results of the study reveal that the rates of oxidative aromatization of 1,4-DHPs are substituent dependent. Thus, the present study is very useful in understanding the metabolism of 1,4-DHP drugs in liver by cytochrome P450 and designing novel drugs as well as modifying the existing drugs for better pharmacokinetic profile.



1,4-Dihydropyridines (1,4-DHPs)[1] have been established as one of the first line drugs for treatment of hypertension because of their promising depressor effect and relatively good tolerability.[2] Felodipine, amlodipine, nifedipine, and nicardipine (Fig. 1) are among the best selling drugs. These drugs act by modulating calcium channel ($Ca^{2+}$) currents.[3] 1,4-DHPs have been extensively studied because of the biological significance of these compounds in the NADH redox process[4] as well as their therapeutic functions for treatment of a variety of diseases, such as cardiovascular disorders, cancer, and AIDS.[5]

Metabolic studies of 1,4-DHP drugs in the human body have shown that these compounds are oxidized to pyridine derivatives by the action of cytochrome P450 in the liver.[6] The oxidation of easily available 1,4-DHPs[7] to the corresponding pyridine derivatives constitutes principal metabolic route in biological systems, as well as provides a facile access to the corresponding pyridine derivatives, which show anti-hypoxic and anti-ischemic activities.[8] The main representative of oxidized 1,4-DHPs is cerivastatin which has found its application in the treatment of atherosclerosis and other coronary deseases.[9] Therefore, oxidative aromatization of 1,4-DHPs has attracted continuing interests of organic and medicinal chemists and a plethora of protocols has been developed.[10–12]

In the course of drug discovery, features such as absorption, distribution, metabolism, and excretion (ADME) are determinant parameters that need to be studied at the earliest stages.[13] In mammals, family of cytochrome P450 are primarily responsible for the oxidative metabolism.[14] Cytochrome P450 is a very large and diverse superfamily of hemoproteins found in all domains of life. Cytochrome P450 uses an excess of both exogenous and endogenous compounds as substrates in enzymatic reactions. The most common reaction catalyzed by cytochrome P450 is a monooxygenase, for example, insertion of one atom of oxygen into an organic substrate (RH) while the other oxygen atom is reduced to water:

$$RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$$

Cytochrome P-450 (P-450) has been utilized for catalytic dehydrogenation of 1,4-dihydropyridines.[15] The active site of cytochrome P450 contains a heme iron center (Fig. 2). The iron is tethered to the P450 protein via a thiolate ligand derived from a cysteine residue. The iron ion in the metalloporphyrins interconverts between several oxidation states (+2, +3, +4, and +6) and executes the redox reactions.[16] This prompted us to utilized human hemoglobin–$H_2O_2$ (HbA–$H_2O_2$)[17] as a biomimetic catalyst system for oxidative aromatization of 1,4-DHPs.

Herein we report biocatalytic activity of human hemoglobin (HbA) for the oxidative aromatization of 1,4-dihydropyridines with $H_2O_2$. We preferred hydrogen peroxide as oxidant over other available oxidizing agent since it is cheap, operationally safe, environmentally friendly, easy to handle and produces only water as a by product, which reduces purification requirements.

HbA is an iron porphyrin metalloprotein and acts as an oxygen carrier in biological system. The commercially available HbA contains approximately 80% of the methaemoglobin (Met Hb). METhemoglobin is an oxygen carrying protein hemoglobin having

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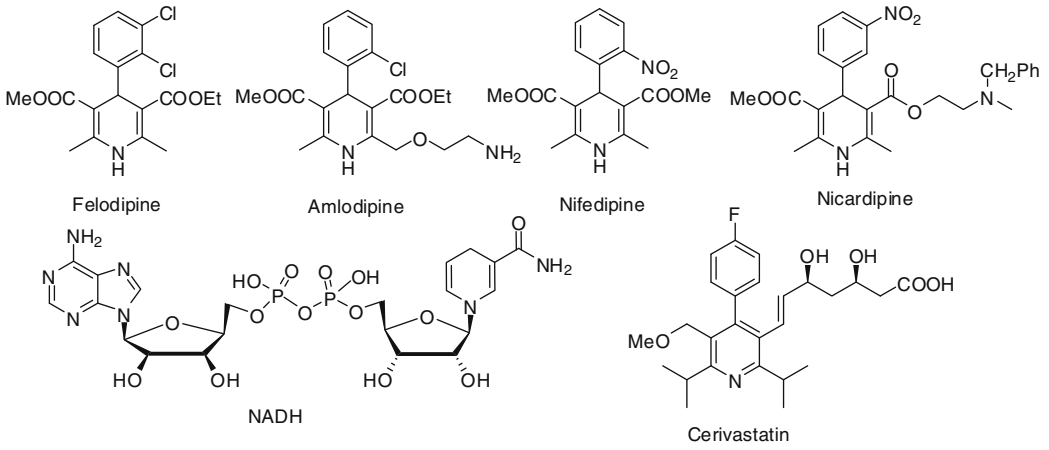



**Figure 1.**

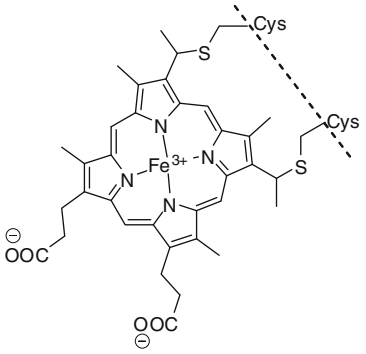



**Figure 2.** Heme group in active site of cytochromes.

$Fe^{3+}$ state of iron in the heme group and is therefore unable to transfer oxygen. Thus, our initial strategy was to convert methemoglobin into oxy-hemoglobin which can efficiently transfer the oxygen. Therefore, we converted commercial human hemoglobin into oxyhemoglobin by the treatment with dithionite in phosphate buffer and subsequently eluted over Sephadex G-25 column to obtain the purified form of oxyhemoglobin (HbAo). The percentage of Oxy Hb of the total HbA was determined from the Absorbance ($A$) at 540 nm and at 576 nm. This equation is based on reported molar absorptivities of oxy-Hb and met-Hb.[18]

$$X = \frac{70A_{540} - 117A_{576}}{169A_{576} - 236A_{540}} \times 100\%$$

In our preliminary experiments 1,4-DHP **1a** was chosen as a model substrate and the reaction was studied by using 30% aqueous hydrogen peroxide with the catalytic amount of purified oxy-Hb (method-I). We observed that the purified oxyhemoglobin oxidised 1,4-DHP quite efficiently and furnished the corresponding pyridine derivative in high yield but this procedure needs extra efforts for purification of the catalyst. We therefore, developed two other methods for the oxidative aromatization (method II and III).

The crude oxyhemoglobin (method II) did not work well for oxidative aromatization of 1,4-DHPs. However, method III was found to be best among three protocols (I, II, and III). The protocol III gave excellent yields of aromatized 1,4-DHP in short reaction times (Fig. 3). No reaction was detected when either hemoglobin or hydrogen peroxide was used alone.

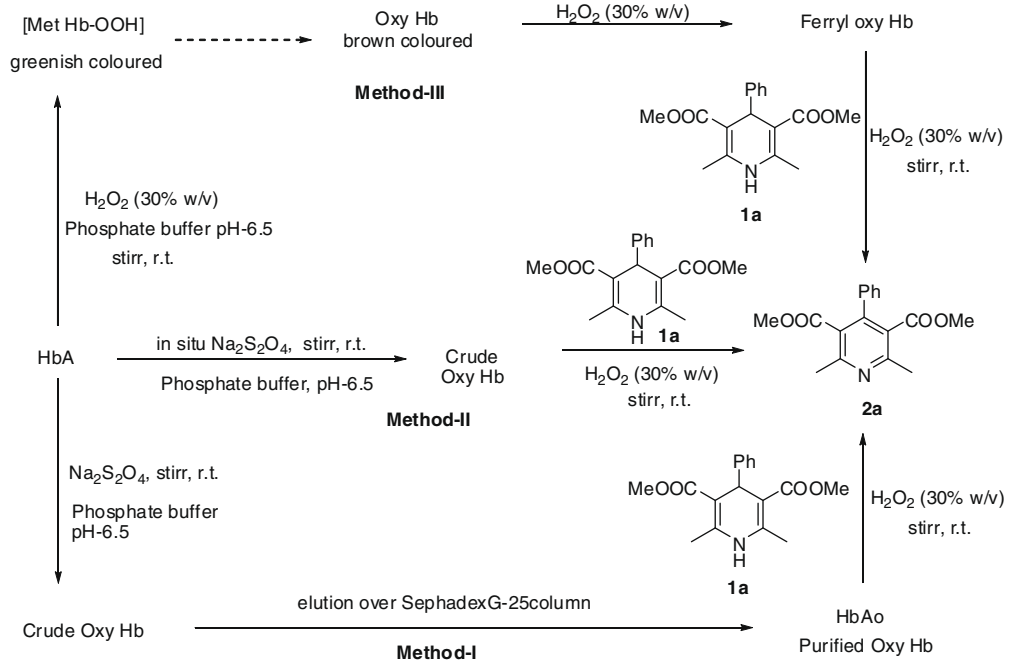



**Figure 3.** Optimization of the protocol for HbA/$H_2O_2$ mediated oxidative aromatization of 1,4-DHPs.

In order to check the feasibility of HbA/$H_2O_2$ catalyzed system in organic medium, we performed the oxidative aromatization in ethanol, acetonitrile, methanol, and $CH_2Cl_2$. Although organic medium were found unsuitable for this biomimetic oxidative aromatization, HbA/$H_2O_2$ system worked quite efficiently in 15% of acetonitrile in phosphate buffer. The results of this study are summarized in Table 1.

The oxidative aromatization of 1,4-DHP **1a** proceeded smoothly at room temperature in 15% acetonitrile in phosphate buffer giving excellent yields of **2a** in 5 h (method III). The presence of acetonitrile was necessary for the reaction to proceed smoothly. Since the reaction gave almost quantitative yields of the products in the presence of 15% acetonitrile in phosphate buffer, we did not study the affects of adding any tension active compounds to the system (Micellar system). An attempt to increase the reaction rate by heating the reaction mixture resulted in decreased yields of **2a**. Upon refluxing the reaction mixture, **2a** was isolated in only 40% yield. Thus, we concluded that stirring the reaction at room temperature was the optimal condition.

Next, we examined the scope of the HbA–$H_2O_2$ mediated oxidative aromatization of 1,4-DHPs (Table 2). A series of 1,4-DHPs were aromatized using the optimized reaction conditions. The reaction proceeded smoothly with various 1,4-DHPs bearing electron rich as well as electron deficient aromatic substituents at C-4. The reaction is quite general and was successfully applied to 1,4-DHPs having aliphatic as well as heteroaromatic substituents at C-4. However when the C-4 of the 1,4-DHP was substituted with benzyl or isopropyl group dealkylation occurred (entries 16 and 17). Benzyl alcohol was isolated as a side product for entry (16) but the corresponding alcohol was not isolated for entry (17). The reaction was quite regioselective as only oxidative aromatization of the 1,4-DHPs occurred. Alcohols were not oxidized in our reaction conditions. We attempted to oxidize various alcohols (ethanol, isopropyl alcohol, and benzyl alcohol) in the present reaction conditions but corresponding oxidized product was not obtained. This indicates that an alcohol present in a side chain of 1,4-DHP should not oxidize under the reaction conditions.

A careful examination of the results of Table 2 revealed that the reaction time for each 1,4-DHP was different. This inspired us to investigate the substituent effect over the rate of oxidative aromatization of 1,4-DHPs. The reactions were performed under protocol III. The test samples were withdrawn at 30 min intervals and HPLC was performed to determine the progress of the reaction (% oxidation). The percentage oxidation was calculated by measuring the AUC of oxidized and unoxidized 1,4-DHP. We studied the effect of substitution at C-2 and C-6 (methyl vs *n*-propyl) (Fig. 4a), C-3 and C-5 (methyl, ethyl, isopropyl, and *tert* butyl esters) (Fig. 4b) and C-4 (H, phenyl, *p*-methoxyphenyl, and *p*-nitrophenyl) (Fig. 4c) of the 1,4-DHPs over the rate of oxidative aromatization. The results of this study are summarized in Figure 4.

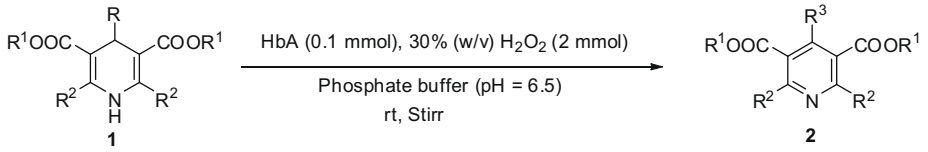
The results of Figure 4a shows that size of $R^1$ at C-2 and C-6 of the 1,4-DHPs affects the rate of oxidative aromatization. With *n*-propyl group (entry 5) rate of aromatization was slow in comparison to methyl group (entry 2). In contrast to it the rate of aromatization increases significantly with increasing the size of ester group. In the case of methyl ester (entry 1) rate of aromatization was slower while with tertiary butyl ester (entry 4) rate of aromatization was faster (Fig. 4b). The rate of aromatization was faster for simple 1,4-DHPs ($R^3$ = H, entry 15). The reaction time was very little affected by the electronic nature of substituent present in

**Table 1**
Optimization of solvent for HbA–$H_2O_2$ mediated oxidative aromatization of **1a**[a]

| Entry | Solvent | Time (h) | Yield of **2a**[b] (%) |
|---|---|---|---|
| 1 | $CH_2Cl_2$ | 12 | 30 |
| 2 | Ethanol | 8 | 60 |
| 3 | Methanol | 8 | 62 |
| 4 | Acetonitrile | 8 | 72 |
| 5 | Phosphate buffer | 8 | 80 |
| 6 | 15% Acetonitrile in phosphate buffer | 5 | 95 |

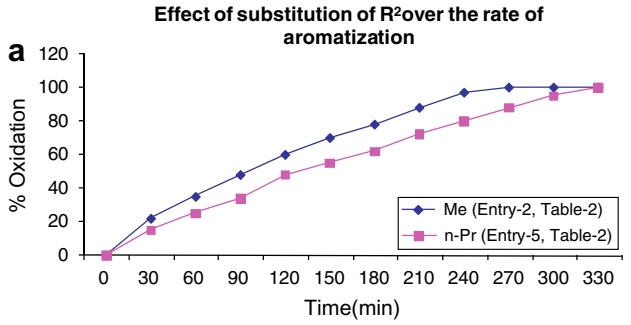
[a] Reaction condition: 1,4-DHP **1a** (1 mmol), ferryl oxy Hb (0.1 mmol), $H_2O_2$ (2 mmol), stir, rt.
[b] Isolated yield.

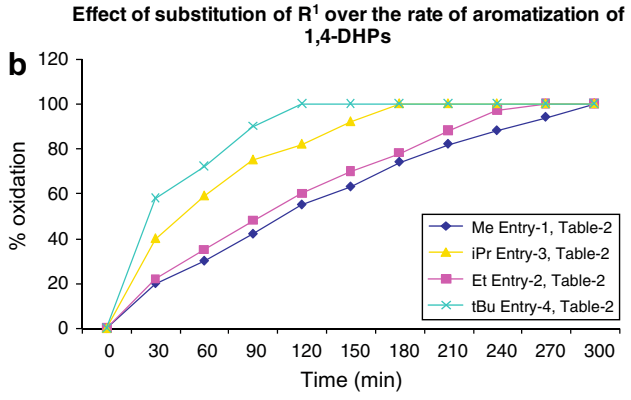
**Table 2**
HbA/$H_2O_2$ catalyzed oxidative aromatization of Hantzsch 1,4-DHPs[a]

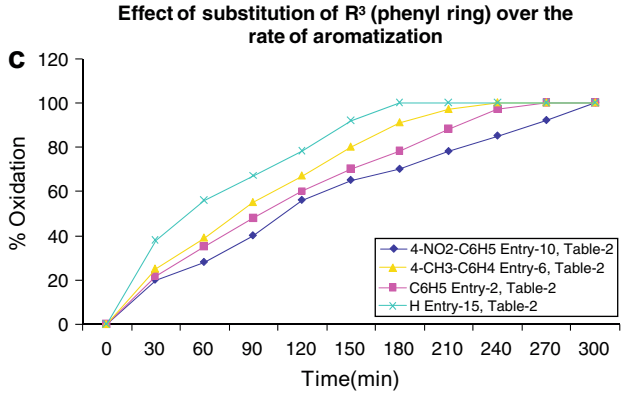



| Entry | R | $R^1$ | $R^2$ | $R^3$ | Product[b] | Time (h) | Yield[c] (%) |
|---|---|---|---|---|---|---|---|
| 1 | $C_6H_5$ | $CH_3$ | $CH_3$ | $C_6H_5$ | **2a** | 5 | 97 |
| 2 | $C_6H_5$ | $CH_2CH_3$ | $CH_3$ | $C_6H_5$ | **2b** | 4.5 | 96 |
| 3 | $C_6H_5$ | $CH(CH_3)_2$ | $CH_3$ | $C_6H_5$ | **2c** | 3 | 98 |
| 4 | $C_6H_5$ | $C(CH_3)_3$ | $CH_3$ | $C_6H_5$ | **2d** | 2 | 95 |
| 5 | $C_6H_5$ | $CH_2CH_3$ | $(CH_2)_2CH_3$ | $C_6H_5$ | **2e** | 5.5 | 96 |
| 6 | 4-$CH_3$–$C_6H_5$ | $CH_2CH_3$ | $CH_3$ | 4-$CH_3$–$C_6H_5$ | **2f** | 4 | 95 |
| 7 | 4-$CH_3O$–$C_6H_5$ | $CH_2CH_3$ | $CH_3$ | 4-$CH_3O$–$C_6H_5$ | **2g** | 4.5 | 96 |
| 8 | 4-Cl–$C_6H_5$ | $CH_2CH_3$ | $CH_3$ | 4-Cl–$C_6H_5$ | **2h** | 5 | 95 |
| 9 | 3-$NO_2$–$C_6H_5$ | $CH_2CH_3$ | $CH_3$ | 3-$NO_2$–$C_6H_5$ | **2i** | 5 | 94 |
| 10 | 4-$NO_2$–$C_6H_5$ | $CH_2CH_3$ | $CH_3$ | 4-$NO_2$–$C_6H_5$ | **2j** | 5 | 96 |
| 11 | $C_6H_5CH{=}CH$ | $CH_2CH_3$ | $CH_3$ | $C_6H_5CH{=}CH$ | **2k** | 5 | 97 |
| 12 | 2-Furyl | $CH_2CH_3$ | $CH_3$ | 2-Furyl | **2l** | 4.5 | 99 |
| 13 | 2-Thenyl | $CH_2CH_3$ | $CH_3$ | 2-Thenyl | **2m** | 4.5 | 98 |
| 14 | $CH_3$ | $CH_2CH_3$ | $CH_3$ | $CH_3$ | **2n** | 3.5 | 95 |
| 15 | H | $CH_2CH_3$ | $CH_3$ | H | **2o** | 3 | 97 |
| 16 | $CH_2C_6H_5$ | $CH_2CH_3$ | $CH_3$ | H | **2o** | 4 | 85 |
| 17 | $CH(CH_3)_2$ | $CH_2CH_3$ | $CH_3$ | H | **2o** | 4 | 80 |

[a] Reaction conditions: 1,4-DHP (1 mmol), HbA (0.1 mmol), $H_2O_2$ (30% w/v, 2 mmol), 15% acetonitrile in phosphate buffer (pH 6.5, 3 ml), stir, rt.
[b] All the products were known and characterized by comparison of their melting points, IR, and $^1H$ NMR spectra with literature.



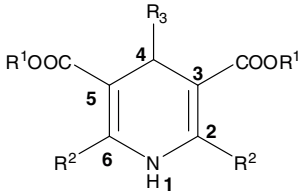
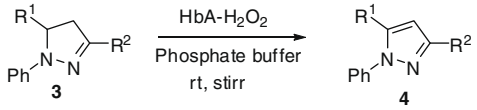




**Figure 4.** Substituent effect over the rate of aromatization of 1,4-DHPs.

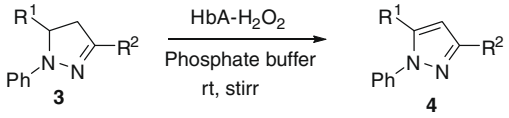
aromatic ring at C-4. However with electron deficient aromatic ring (4-nitrophenyl, entry 10) aromatization was slower (Fig. 4c).

We further examined the scope of the HbA–$H_2O_2$ catalyst system for oxidative aromatization of pyrazolines (Scheme 1, Table 3). Using the optimized protocol III, treatment of 1,2,3-tri-




**Scheme 1.**

**Table 3**
Scope of the HbA–$H_2O_2$ catalyst system for oxidative aromatization of 1,2,3-trisubstituted pyrazolines[a]




| Entry | $R^1$ | $R^2$ | Product[b] | Yield[c] (%) |
|---|---|---|---|---|
| 1 | $C_6H_5$ | $C_6H_5$ | **4a** | 80 |
| 2 | 4-$MeOC_6H_4$ | $C_6H_5$ | **4b** | 85 |
| 3 | 4-$NO_2C_6H_4$ | $C_6H_5$ | **4c** | 86 |
| 4 | 4-$ClC_6H_4$ | $C_6H_5$ | **4d** | 84 |
| 5 | 2-Furyl | 4-$CH_3$–$C_6H_4$ | **4e** | 83 |
| 6 | 2-Thenyl | 4-$ClC_6H_4$ | **4f** | 78 |

[a] Reaction conditions: Pyrazoline **3** (1 mmol), HbA (0.1 mmol), $H_2O_2$ (30% w/v, 2 mmol), 15% acetonitrile in phosphate buffer (pH 6.5, 3 ml), stir, rt, 5 h.
[b] All the products were known and characterized by comparison of their melting points, IR, and $^1H$ NMR spectra with literature.
[c] Isolated yield.

substituted pyrazoline derivatives with HbA–$H_2O_2$ led to the formation of corresponding pyrazoles in high yields within 4–5 h at room temperature.

A detail study for mechanistic aspect of HbA–$H_2O_2$ mediated oxidation of 1,4-DHPs and pyrazoline is currently underway in our group. All these results will be published in a separate paper in near future.

In conclusion, we have reported an efficient biomimetic oxidative aromatization of 1,4-DHPs and 1,2,3-trisubstituted pyrazolines catalyzed by HbA/$H_2O_2$ in 15% acetonitrile–phosphate buffer. The present study provides useful correlation between substituent effect and the rate of aromatization of 1,4-DHPs. Such correlations are important in designing new drugs, modifying the existing drugs for better pharmacokinetic profile and understanding metabolism of 1,4-DHPs based drugs in liver by cytochrome P450 enzymes.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.056.

## References and notes

1. (a) Mauzeral, D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2261; (b) Abeles, R. J.; Hutton, R. F.; Westheimer, F. H. *J. Am. Chem. Soc.* **1957**, *79*, 712.
2. Ana, B. B.; Rosa, M. A.; Rosa, M. J.; Wolfgang, W. *Forensic Sci. Int.* **2006**, *156*, 23.
3. (a) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 775; (b) Bossert, F.; Vater, W. *Med. Res. Rev.* **1989**, *9*, 291.
4. Bocker, R. H.; Guengerich, F. P. *J. Med. Chem.* **1986**, *29*, 1596.
5. (a) Triggle, D. J.; Langs, D. A.; Janis, R. A. *Med. Res. Rev.* **1989**, *9*, 123; (b) Kawase, M.; Shah, A.; Gaveriya, H.; Motohashi, N.; Sakagami, H.; Varga, A.; Molnar, J. *Bioorg. Med. Chem.* **2002**, *10*, 1051; (c) Hilgeroth, A. *Mini-Rev. Med. Chem.* **2002**, *2*, 235; (d) Max, I. T.; Zhang, J.; Weglicki, W. B. *Pharmacol. Res.* **2002**, *45*, 27.
6. (a) Guengerich, F. P.; Brian, W. R.; Iwasaki, M.; Sari, M. A.; Baarnhielm, C.; Berntsson, P. *J. Med. Chem.* **1991**, *34*, 1838; (b) Böcker, R. H.; Guengeric, F. P. *J. Med. Chem.* **1986**, *28*, 1596; (c) Kudo, S.; Okumura, H.; Miyamoto, G.; Ishizaki, T. *Drug Metab. Dispos.* **1999**, *27*, 303.
7. (a) Sabitha, G.; Reddy, G. S. K. K.; Reddy, C. S.; Yadav, J. S. *Tetrahedron Lett.* **2003**, *44*, 4129; (b) Zolfigol, M. A.; Safaiee, M. *Synlett* **2004**, 827; (c) Kumar, A.; Maurya, R. A. *Synlett* **2008**, 883.
8. Khadikar, B.; Borkat, S. *Synth. Commun.* **1998**, *28*, 207 and references cited therein.

9. Bischhoff, H.; Angerbauer, R.; Bender, E.; Bischoff, J. A.; Petzinna, D.; Pfitzner, J.; Porter, M. C.; Schmidt, D.; Thomas, G. *Atherosclerosis* **1997**, *135*, 119.
10. (a) Sausins, A.; Duburs, G. *Heterocycles* **1988**, *27*, 291; (b) Chennot, T.; Eisner, U. *J. Chem. Soc., Perkin Trans. 1* **1975**, 926; (c) Vanden Eynde, J. J.; Orazio, R. D.; Van Haverbeke, Y. *Tetrahedron* **1994**, *50*, 2479; (d) Pfister, J. R. *Synthesis* **1990**, 689; (e) Yadav, J. S.; Subba Reddy, B. V.; Sabitha, G.; Reddy, G. S. K. K. *Synthesis* **2000**, *11*, 1532; (f) Itoh, T.; Nagata, K.; Matsuya, Y.; Miyazaki, M.; Ohsawa, A. *J. Org. Chem.* **1997**, *62*, 3582; (g) Mashraqui, S. H.; Karnik, M. A. *Synthesis* **1998**, 713; (h) Varma, R. S.; Kumar, D. *Tetrahedron Lett.* **1999**, *40*, 21; (i) Ko, K.-Y.; Kim, J.-Y. *Tetrahedron Lett.* **1999**, *40*, 3207; (j) Anniyappan, M.; Muralidharan, D.; Perumal, P. T. *Tetrahedron* **2002**, *58*, 5069; (k) Zhu, X.-Q.; Zhao, B.-J.; Cheng, J. P. *J. Org. Chem.* **2000**, *65*, 8158.
11. (a) Lopez-Alarcon, C.; Nunez-Vergara, L. J.; Sturm, J. C.; Squella, J. A. *Electrochim. Acta* **2003**, *48*, 505; (b) Arguello, J.; Nunez-Vergara, L. J.; Sturm, J. C.; Squella, J. A. *Electrochim. Acta* **2004**, *49*, 4849.
12. (a) Mashraqui, S. H.; Karnik, M. A. *Tetrahedron Lett.* **1998**, *39*, 4895; (b) Nakamichi, N.; Kawashita, Y.; Hayashi, M. *Org. Lett.* **2002**, *4*, 3955; (c) Nakamichi, N.; Kawashita, Y.; Hayashi, M. *Synthesis* **2004**, 1015.
13. Ansede, J. H.; Thakker, D. R. *J. Pharm. Sci.* **2004**, *93*, 239.
14. de Montellano, P. R.; de Voss, J. J. *Nat. Prod. Rep.* **2002**, *19*, 477.
15. Guengerich, F. P.; Biickerg, R. H. *J. Biol. Chem.* **1988**, *263*, 8168.
16. Filipan-Litvić, M.; Litvić, M.; Vinkovic, V. *Bioorg. Med. Chem.* **2008**, *16*, 9276.
17. Kumar, A.; Akanksha *Tetrahedron Lett.* **2007**, *48*, 7857.
18. Riggs, A. In *Methods in Enzymology*; Antoninie, E., Rossi-Bernardi, L., Chiancone, E., Eds.; Hemoglobins; Academic Press: New York, 1981; Vol. 76, pp 22–23.