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# Synthesis of potential drug metabolites by a modified Udenfriend reaction

Roger Slavik [†], Jens-Uwe Peters [*], Rudolf Giger [‡], Markus Bürkler, Eric Bald

*Discovery Chemistry, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*

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ABSTRACT

Several drugs (clozapine, chlorpromazine, imipramine, buspirone, diltiazem, and propranolol) were subjected to modified Udenfriend conditions ($Fe^{2+}$/$Mn^{2+}$/EDTA/ascorbic acid/$O_2$). From each reaction, one to four oxidation products were obtained in 1–8% overall yield. Many of these products (9 out of 14) have been reported to be metabolites of the parent drugs in vivo. The products resulted mainly from aromatic hydroxylation, and are not readily accessible by conventional synthesis. Thus, the described reaction may be useful in drug discovery whenever a facile synthetic access is more important than high yields (e.g., for a fast derivatisation of compounds or the preparation of metabolites). Poorly water-soluble compounds cannot be converted, which is an important limitation of this method.



Oxidation reagents and reaction systems, which mimic Cytochrom-P450 (CYP) mediated phase I metabolism,[1] have been an area of active research.[2] Such reagents are often based on a 'single oxygen donor' (such as $H_2O_2$), or molecular oxygen as an oxidative reagent, a transition-metal catalyst (e.g., $Fe^{2+}$), and frequently, a metal-complexing ligand; electrochemical oxidations are also known.[3] Unless an intentionally simple model compound is used, these reagents typically give rise to a range of oxidation products, which are reminiscent of the oxidation patterns observed in CYP metabolism. For instance, the oxidation of phencyclidine[4] or cimetidine[5] with Fenton's reagent ($Fe^{2+}$/$H_2O_2$) yields several metabolite-like products. Predictably selective reagents have also been discovered recently.[6] Similarly, several (enantio-) selective methods for the transition-metal catalysed epoxidation of alkenes are established synthetic tools in organic synthesis.[7]

Potentially, biomimetic oxidations providing a reasonable number of oxidation products from single substrates would be highly desirable for drug discovery, because they might provide a fast access to metabolites, but would also be useful for a fast derivatisation of compounds in the early hit-to-lead phase of a discovery project. Low yields would be acceptable as long as such reactions provide a fast access to small amounts (~5 mg) of target compounds for pharmacological characterisation or as references for PK studies. Nevertheless, biomimetic oxidations have not yet found widespread use in industrial research, possibly because a straightforward, general use for preparative purposes may not be obvious from the literature: many papers discuss the study of specific oxidations with simple model compounds, the use of optimised conditions for the synthesis of specific metabolites, or oxidations without a (preparative) isolation of the products (e.g., for in vitro toxicity tests, or for spectroscopic identifications).[3] Instead, industrial researchers frequently rely on liver preparations or recombinantly expressed CYPs for the synthesis of metabolites.[8] Liver preparations, for example, microsomes, are, however, costly and available only in small quantities, which limits the amount of obtained metabolites to the microgram range. The synthesis via recombinant CYPs gives larger amounts of metabolites, but is labour-intensive and time-consuming; moreover, the relevant CYPs have to be identified beforehand in metabolite identification studies.

The Udenfriend reaction system ($Fe^{2+}$, EDTA, ascorbic acid, $O_2$)[9] has been explored as a model of hepatic metabolism,[10] particularly for aromatic hydroxylation (for mechanistic studies and discussion, see Ref.[11]). To investigate whether such a biomimetic oxidation might be an alternative to the above-mentioned biotechnology-based oxidations, we subjected marketed drugs to a $Fe^{2+}$/$Mn^{2+}$ variant of the Udenfriend reaction system (for details see note[12]).

Clozapine, chlorpromazine, imipramine, buspirone, diltiazem, and propranolol were oxidised under these conditions until analytical HPLC indicated a complete consumption of the substrate (Scheme 1). The HPLC chromatogram suggested clean reactions, with no remaining starting material and a reasonably small number of product peaks. However, most of the starting material seems to have been converted to a black, tarry product in all of these reactions. This insoluble product is easily removed by filtration,

* Corresponding author. Tel.: +41 61 68 82636.
*E-mail addresses:* slavikr@student.ethz.ch (R. Slavik), jens-uwe.peters@roche.com (J.-U. Peters), giger.mcc@gmail.com (R. Giger).
† Present address: Steinbock Apotheke, Quaderstrasse 16, CH-7000 Chur, Switzerland.
‡ Present address: GIGER MedChem Consulting, Eichenweg 20, CH-4132 Muttenz, Switzerland.

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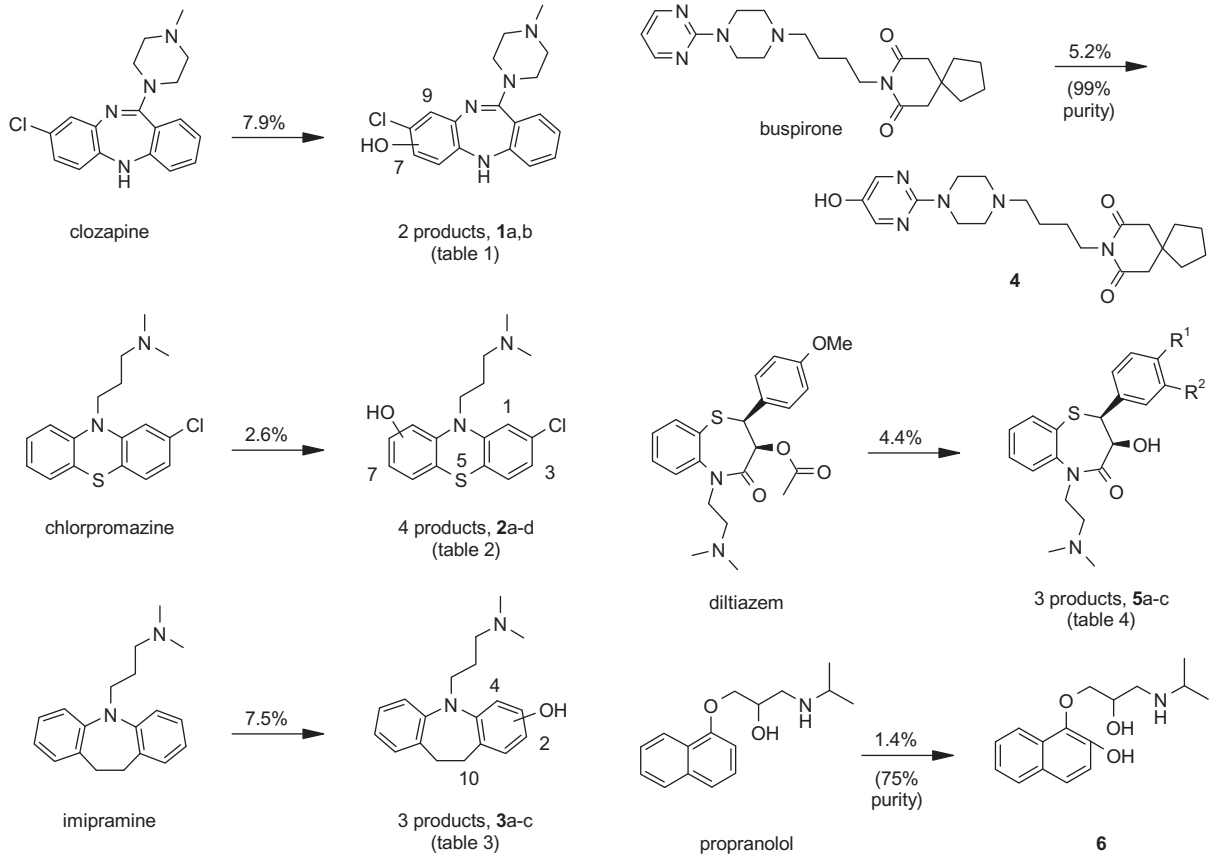


**Scheme 1.** Reagents and conditions: $O_2$ (ambient pressure), $FeSO_4$, $Mn(OAc)_2$, EDTA, ascorbic acid, aqueous buffer (pH 4), 2 h 45 °C. The given yields are overall yields of isolated products; yields are purity-corrected.[19]

whereas the desired oxidation products are readily separated from the other components of the reaction mixture by an adjustment of the pH to ~8.5, and extraction with organic solvent. The oxidation products were then isolated by preparative reverse-phase HPLC with a standardised solvent gradient. Compounds were characterised by a variety of NMR methods[13] and MS. The results for each compound (Scheme 1) are discussed below:

*Clozapine* (Table 1): the two isolated oxidation products, **1a** and **1b**, are also observed as (relatively minor) human metabolites.[14] The major human metabolites, the N-demethylation and N-oxidation products of clozapine,[15] were not formed in our reaction system; furthermore, the de-chlorination/hydroxylation product, which is another minor human metabolite,[16] was not found. Products **1a** and **1b** may be formed in vivo via a clozapine nitrenium ion as a reactive metabolite.[17,18]

*Chlorpromazine* (Table 2): four oxidation products, **2a–d**, were isolated. Three of the products are hydroxylated in an activated aromatic position, whereas the fourth product is a sulfoxide. Chlorpromazine forms numerous metabolites in man; **2c** and **2d** are major metabolites which may contribute to the therapeutic efficacy of chlorpromazine.[20]

*Imipramine* (Table 3): aromatic hydroxylation took place in the activated 2- and 4-position. The yield of **3b** (4-OH) was somewhat higher than that of **3a** (2-OH); **3b** is also a major metabolite in humans.[21] Additionally, a benzylic methylene unit was oxidised (**3c**), which is the only example for an aliphatic hydroxylation among the investigated compounds (apart from demethylation, which may involve $CH_3$ hydroxylation as a mechanistic step). Compound **3c** is also found as a human metabolite.[22]

*Buspirone*: the aromatic hydroxylation product **4** was obtained as the only product in relatively good (5.2%) yield and high (99%) purity. Compound **4** is one of the numerous buspirone metabolites, which are observed in vivo.[23] The important buspirone metabolite 1-pyrimidinylpiperazine, which is believed to contribute to buspirone's anxiolytic effects and its duration of action,[24] was not found in the reaction mixture.

**Table 1**
Clozapine oxidation products

| Compd | | Yield (%) | Purity[19] (%) |
|---|---|---|---|
| **1a** | 7-OH | 4.93 | 84 |
| **1b** | 9-OH | 2.98 | 71 |

**Table 2**
Chlorpromazine oxidation products

| Compd | | Yield (%) | Purity[19] (%) |
|---|---|---|---|
| **2a** | 1-OH | 0.40 | 86 |
| **2b** | 3-OH | 0.84 | 80 |
| **2c** | (5) S=O | 0.75 | 100 |
| **2d** | 7-OH | 0.58 | 81 |

**Table 3**
Imipramine oxidation products

| Compd | | Yield (%) | Purity[19] (%) |
|---|---|---|---|
| **3a** | 2-OH | 1.46 | 84 |
| **3b** | 4-OH | 3.47 | 96 |
| **3c** | 10-OH | 2.56 | 89 |

*Diltiazem* (Table 4): the ester motif of the parent compound was hydrolysed to the alcohol in all three isolated products. Whereas **5a** was merely the hydrolysis product (i.e., no oxidation occured), **5b** and **5c** were additionally hydroxylated or O-de-methylated, respectively. Compound **5a**[25] and **5c**[26] are also human metabolites of diltiazem.

*Propranolol*: the *ortho*-hydroxylated **6** was the only isolated product; the main in vivo metabolite, resulting from a hydroxylation in the *para*-position of the N substituent,[27] was not found. Moreover, the formation of **6** was relatively inefficient (1.4% yield, 75% purity). The observed regioselectivity may be explained by a metal-chelating and thus *ortho*-directing effect of the propranolol side chain.

All products were isolated by a standardised reverse-phase HPLC protocol in a purity of >70% (analytical HPLC with UV detection at $\lambda = 254$ nM), a purity which may be regarded as sufficient for a preliminary in vitro pharmacological characterisation. All experiments were performed only once, and no attempts were made to improve the yields or purities.

The majority of the isolated oxidation products (10 out of 14) are aromatic hydroxylation products. This preference for aromatic hydroxylation may add to the attractiveness of this methodology, because such hydroxylation products are usually difficult to obtain via the established synthetic methods for the parent compounds. In contrast, N-oxides and N-desalkylation metabolites (which are usually easy to synthesize via established routes) were not observed, likely because the protonation of basic alkyl-amines under the acidic reaction conditions protects such motifs from N-oxidation and oxidative desalkylation.

*Other compounds* (Fig. 1): the attempted oxidation of diazepam and gemfibrozil failed due to the poor solubility of these compounds in the acidic, aqueous reaction medium. Pre-dissolution in acetone, or the addition of acetone or methanol as a co-solvent brought no improvement. Amantadine gave no oxidation products, despite good solubility; HPLC analysis indicated that the starting material remained unchanged. Interestingly, phase I (oxidative) metabolism plays also no significant role in vivo, and amantadine is mainly excreted unchanged renally.[28] Verapamil gave a mixture of regioisomeric O-de-methylation products, which was unseparable under our standard HPLC conditions (13.2% isolated yield). O-de-methylation is also the major metabolic pathway of verapamil in vivo.[29]

**Table 4**
Diltiazem oxidation/hydrolysation products

| Compd | $R^1$ | $R^2$ | Yield (%) | Purity[19] (%) |
|---|---|---|---|---|
| **5a** | OMe | H | 1.75 | 77 |
| **5b** | OMe | OH | 1.8 | 98 |
| **5c** | OH | H | 0.81 | 82 |

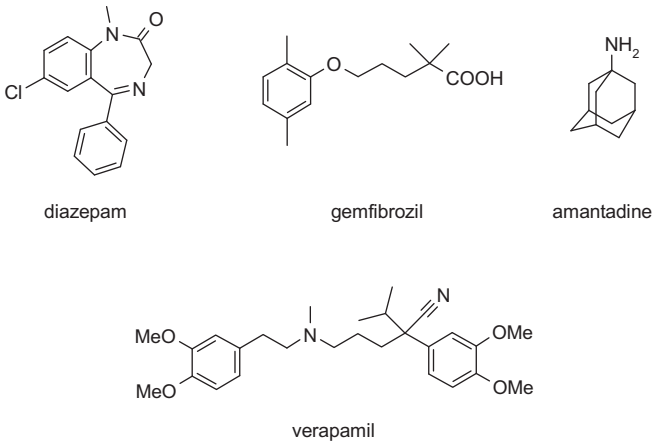


**Figure 1.** Other drugs subjected to the described conditions. Diazepam and gemfibrazil were not sufficiently soluble, amantadine was inert to the oxidation conditions, verapamil gave unseparable products (see text).

Additional experiments were performed to compare the modified Udenfriend conditions with the Fenton conditions, and the original Udenfriend conditions. Oxidation of clozapine and propranolol under both Fenton conditions described in Ref.[30] yielded numerous products, but only traces of mono-hydroxylation products, as indicated by HPLC/MS. Oxidation of clozapine under the original Udenfriend conditions[9b] gave an isolated yield of 3.0% (94% purity) of **1b**, but no **1a**, whereas propranolol was not converted at all under these conditions within 4.5 h. Thus, at least for clozapine and propranolol, the modified Udenfriend conditions gave superior results in the preparation of metabolite-like products.

The oxidation products **1a**–**4**, **5b**, and **6** should be more susceptible to oxidation than their parent compounds, due to the increased electron-density of the hydroxylated aromatic rings. The formation of black side products may be an indication of substrate over-oxidation and subsequent polymerisation.[31] Thus, reaction times might be optimised to minimise over-oxidation, and thus to maximise the yield of the desired mono-oxidation products. We monitored the oxidation of some substrates by analytical HPLC in time-course experiments, and indeed found optimal reaction times with a maximal relative peak area of the desired product(s) in the HPLC chromatogram (clozapine: 1 h; chlorpromazine: 1.5–2.5 h; imipramine: 2 h; buspirone: 1–2.5 h).[32] We also noted that the reaction rate is very dependent on the set-up of the reaction. For instance, passing oxygen through a glass frit into the reaction mixture leads to much faster reaction times than the use of a capillary. Thus, the optimal reaction time depends on the substrate and the reaction set-up and can be determined case-by-case.

In conclusion, the described modified Udenfriend reaction may be useful to quickly access oxidation products, in particular hydroxylation products, of drug-like compounds. The method may be used in situations were a facile synthetic access is more important than high product yields, for example, for fast derivatisation in the early stages of drug discovery, or for providing small amounts of metabolites with no established or difficult synthetic access. The described conditions are only applicable to compounds which are sufficiently soluble in the aqueous acidic reaction medium. This may constitute a limitation for a broad application in drug discovery, as contemporary discovery projects often deal with poorly water-soluble compounds. Similar oxidation systems, which use organic solvents, appear, therefore, worthy of further investigations.

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31. Black, tarry products were also observed in the attempted oxidation of propranolol under the original Udenfriend conditions, which gave no conversion of propranolol; thus, the observed polymeric products may originate at least in part from other components of the reaction system, for example, ascorbic acid.
32. We also attempted to minimize over-oxidation by using the described modified Udenfriend procedure, however with lower amounts (one third) of reagents, and a limited reaction time (2 h). Under these conditions, clozapine gave, after incomplete conversion, **1a** in 1.5% yield (62% purity), and **1b** in 1.0% yield (100% purity), whereas no oxidation was observed for propranolol.