Electrooxidation Potential as a Tool in the Early Screening for New Safer Clozapine-like Analogues

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The chemical modification of clozapine (**1**) has permitted the finding of new analogues, e.g., olanzapine (**2**), quetiapine (**3**), 5-(4-methylpiperazin-1-yl)-8-chloropyrido[2,3-*b*][1,5]benzoxazepine fumarate (**9**), with a clinical or psychopharmacological profile similar to that of clozapine. However, when developing new derivatives, the designers are discouraged by the development of clozapine-induced agranulocytosis. Different researchers have raised the role played by the oxidizability of the molecule in such a deleterious effect. In the present paper, we examined the oxidation profile (direct scavenging abilities, efficacy in inhibiting lipid peroxidation, and electrooxidation potential) of newly developed methoxy and trifluoromethylsulfonyloxy analogues related to clozapine, some of them being described as putative antipsychotic. The oxazepine derivative **7**, unlike the other diazepine derivatives (**6**, **10**−**12**), was not readily oxidized. Using a statistical predictive model for hematotoxicity previously described, **7** was found in the cluster of potentially nontoxic compounds while diazepine derivatives **6** and **10**−**12** were classified as potentially toxic compounds. Among these original compounds, **7**, which presents a preclinical clozapine-like profile and a low sensitivity to oxidation, could be a promising antipsychotic candidate with low side effects. Considering the tricyclic derivatives examined so far, some elements of structure−oxidation relationship (SOR) might be pointed out. Regarding the nature of the tricyclic ring substituent, from the most to the least sensitive to oxidation, the sequence was as follows: HO > Cl > CH_3O > CF_3SO_2O. The nature of the tricyclic ring influenced also the sensitivity to oxidation; the diazepine moiety appeared to be the most reactive ring compared to oxa- and thiazepine congeners. These parameters could be advantageously integrated in the early design of new safer clozapine-like analogues.

Introduction

During the past decade, the development of new drugs to replace clozapine (**1**) has been an intense challenge. The main reason is that clozapine, although possessing a unique clinical profile,[1] induces hematological disorders and currently remains restricted to some categories of psychotic patients with a costly blood monitoring. Different hypotheses to explain the pharmacological and clinical profile of clozapine, such as a high 5-HT_2/D_2 receptor pK_i ratio[2] and a selectivity for either the D_3 or D_4 dopaminergic receptor,[3,4] were proposed and retained to develop new series of active compounds. Several molecules were recently introduced in the clinic for the treatment of schizophrenia, namely olanzapine (**2**), quetiapine (**3**), risperidone (**4**), and sertindole (**5**) (Figure 1). However, none of them has been shown to be as effective as clozapine in neuroleptic-resistant patients. Moreover, these new drugs still have a high incidence of side effects.

Parallel to our studies on pyridobenzazepine derivatives,[5−7] Wikström and co-workers developed a new series of tricyclic derivatives related to clozapine.[8,9] The main modification of the clozapine structure was the introduction of a trifluoromethylsulfonyloxy (triflate) group in replacement of the classical halogen (chloro or fluoro group). The halogen substituent in different diarylazepines with antipsychotic properties may be considered an important structural element for recognition in the drug−receptor interaction. The influence may be related not only to the electron-withdrawing effect but also to the increased lipophilicity. These authors postulated that an aromatic triflate group attenuates oxidative metabolism in comparison with a hydroxy or methoxy group due to its electron-withdrawing effect.[8,9] Compound **6** was recently described as a putative atypical antipsychotic.[8] Following this first series, several triflate analogues of dibenzazepines, including not only the diazepine but also the oxazepine

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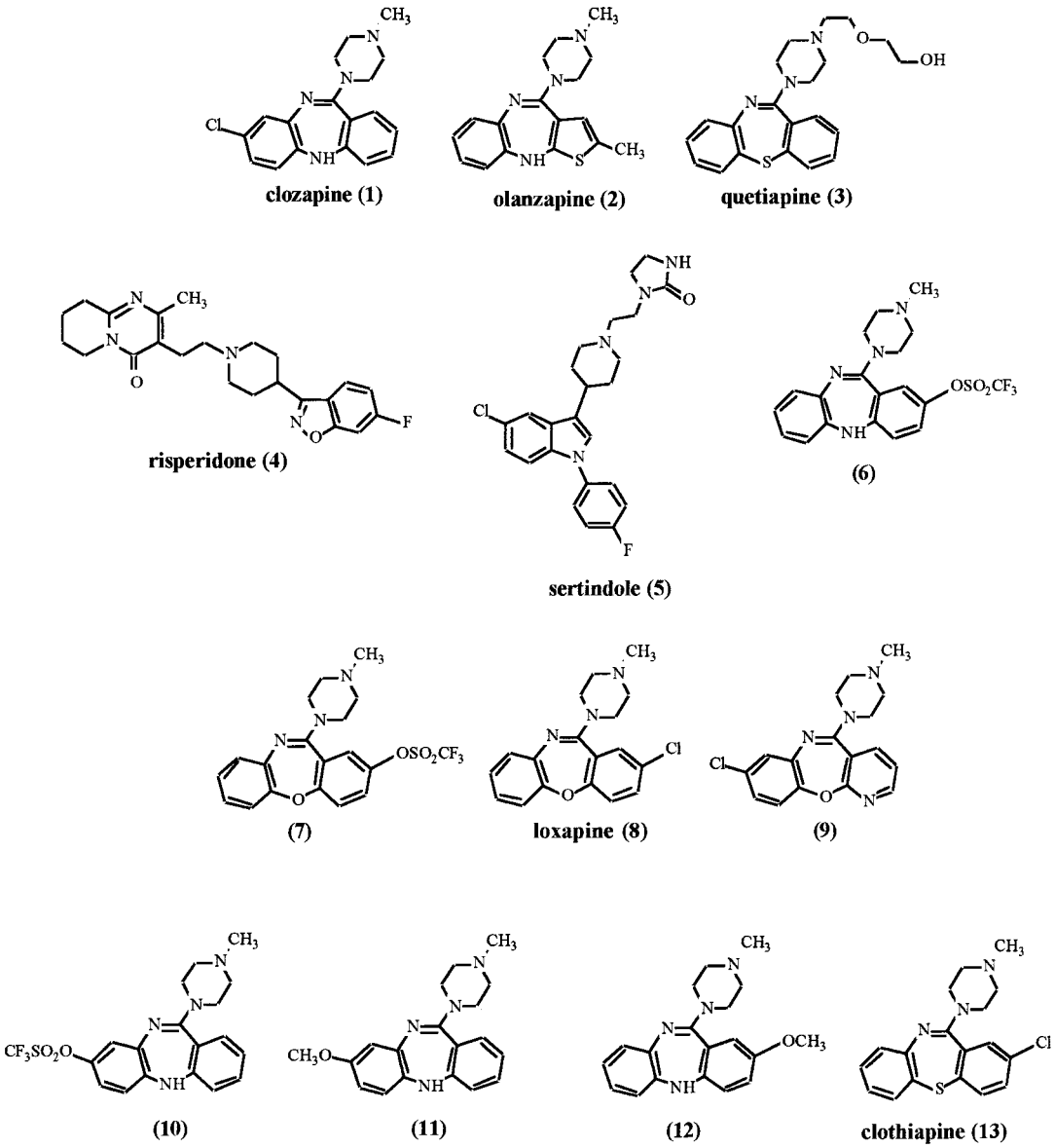

Figure 1. Chemical structure of methoxy and trifluoromethylsulfonyloxy dibenzazepine derivatives and antipsychotic reference molecules.

and thiazepine series, were synthesized. Among them, some 2-triflate analogues also presented a promising clozapine-like profile such as compound **7**.[9]

When developing structurally related templates, the development of clozapine-induced agranulocytosis remains in the drug designer's mind. The main problem is that the exact mechanism(s) of this blood dyscrasia remain(s) unknown.[10,11] In the context of drug development, it is crucial to detect as soon as possible some potential toxicological problems with a new molecule in order to avoid the death of patients and also to avoid sacrificing many animals during the different phases of drug development. The recent history of remoxipride remains freshly present in mind.[12,13]

In this context, the in vitro studies we performed for several years could be an interesting approach in order to eliminate as soon as possible a potentially toxic compound. Effectively, an in vivo model able to detect potential drug-induced hematotoxicity has not yet been reported. This is due to the fact that agranulocytosis is a highly individualized and unexpected reaction to specific drugs.[14,15] Hematological toxicity associated with anticancer drugs is predictable, but many other drugs induce unpredictable idiosyncratic reactions.

Based on Fischer's work,[16] reporting a possible correlation between the clozapine-induced free radical formation and clozapine-induced agranulocytosis, many studies were published in order to identify the possible mechanism by which clozapine induces these hematological side effects.[10,11,17,18] In 1995, we reported the differential sensitivity to peroxidase oxidation of dibenzazepines such as clozapine, loxapine, and clothiapine.[19] In 1997, Uetrecht et al. suggested that clozapine and analogues containing a nitrogen bridge between the two aromatic rings are oxidized by granulocytes or HOCl to a reactive metabolite that irreversibly binds to cells which might be responsible for clozapine-induced agranulocytosis.[18] More recently, we described a combination of several in vitro oxidation methods that might be useful in detecting, as soon as possible in the development of a new drug, a potential hematological toxicity.[20] By multivariate analysis (cluster analysis), we were able

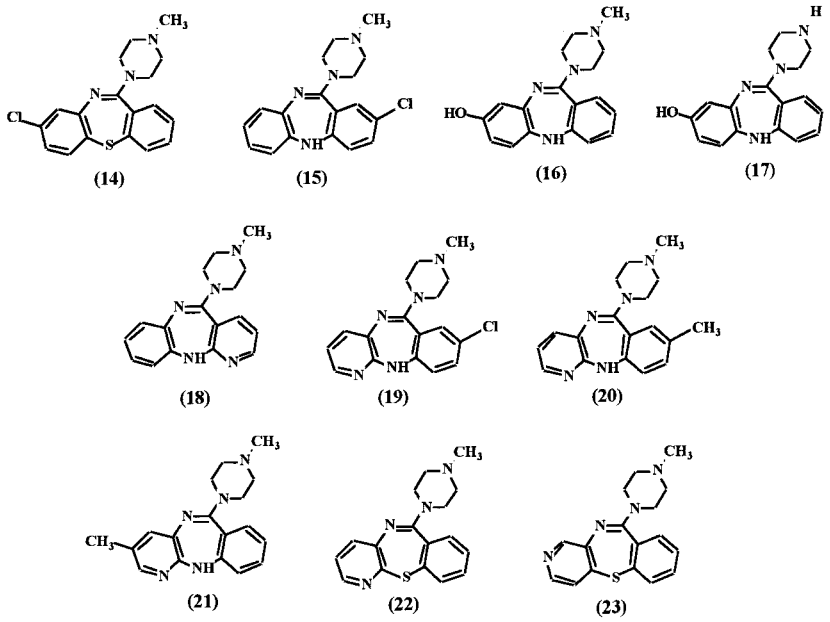

Figure 2. Chemical structure of tricyclic derivatives.

to point out that the electrooxidation potential was the most important parameter in discriminating between safe and unsafe reference compounds.[20]

In the present work, we examined the oxidation profile of several new methoxy and triflate analogues of clozapine. The predicted risk of these original compounds to generate hematological disorders was estimated using our previously described model.[20] Finally, by increasing the number of molecules tested in these models (Figure 2), it might be possible to define a structure−oxidation relationship (SOR) that could be ultimately useful in developing new clozapine-like analogues.

Biochemical and Electrochemical Results

Horseradish Peroxidase (HRP)-Catalyzed Oxidation. Two enzymatic systems were investigated: (I) HRP/GSH (ESR1), initially described by Fischer et al.,[16] and (II) HRP/H_2O_2/GSH (ESR2). Direct detection of clozapine radical with ESR spectroscopy was impossible presumably due to its high reactivity. Fortunately, free radical formation can also be demonstrated by thiyl pumping previously reported.[21] Thiyl pumping involves the catalytic formation of a thiyl radical, which can be detected by spin trapping techniques. Thus, in the presence of DTPA and DMPO, a radical trapping agent, the typical spectrum corresponding to the DMPO/glutathione thiyl radical adduct is characterized by a four-line ESR signal, observed in both systems. This adduct resulted in a distinctive ESR spectrum (a^N (coupling constant of nitrogen) = 15.4 G and a^{H}_{β} (coupling constant of hydrogen) = 16.2 G) with hyperfine splittings similar to those previously reported.[22] The spectrum obtained for each compound (Figure 3), tested at 0.1 mM, was transformed mathematically. This was achieved with a double-integration of each ESR signal using the Bruker PC program. The total area under the ESR curve is proportional to the amount of paramagnetic species in the sample.

The sequence of oxidation in the first system (ESR1) from the most sensitive compound to oxidation to the least could be described as follows: **1** > **8** > **6** > **12** > **11** > **9** > **7** > **10** (Table 1).

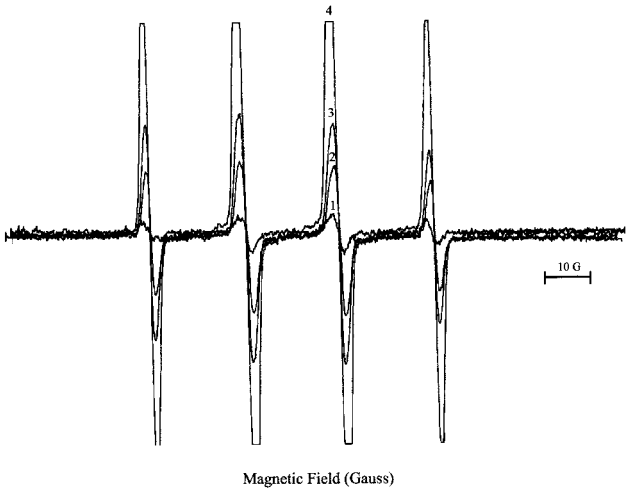

Figure 3. Typical ESR spectrum of DMPO−GSH thiyl radical adduct generated by the HRP/GSH/H_2O_2 system. Spectrum 1 corresponds to the complete system: 25 mg/mL HRP, 10 mM GSH, 1 mM H_2O_2, 0.5 mM DTPA, and 100 mM DMPO in phosphate buffer, pH 7.5 (without drug addition). Spectra 2−4 show the influence of a similar concentration (0.1 mM) of **7**, **11**, and clozapine (**1**) respectively on the intensity of the DMPO−GSH thiyl radical adduct. The instrumental conditions were as follows: microwave power, 20 mW; receiver gain, 2×10^4; time constant, 164 ms; time conversion, 40.96 ms; center field, 3480 ± 50 G; and number of scans, 3.

In the presence of H_2O_2 (ESR2), the sequence of peroxidase oxidation presented some differences compared to the first experiment. The most reactive compound was **1** followed by **12** > **6** > **9** > **11** > **10** > **8** > **7** (Table 1, Figure 3).

Lipid Peroxidation System. The oxidation of an emulsion of linoleic acid by γ-irradiation generates various degradation products. Among them, malondialdehyde (MA) as a breakdown product serves as a convenient index for determining the extent of the peroxidation reaction. In presence of 2-thiobarbituric acid (TBA), MA reacts to give a red species absorbing

Table 1. Experimental Results Obtained with the Five Procedures for Methoxy and Trifluoromethylsulfonyloxy Analogues in Comparison with Clozapine (**1**), Loxapine (**8**), and **9**

drug	ESR1[a]	ESR2[a]	LIPO[b]	OXID4.7[c]	OXID7.4[c]
vehicle	3.8	4.1	100		
1	9.4	42.2	52	450	375
6	5.6	10.9	82	625	525
7	4.3	5.2	66	1055	975
8	5.8	6.5	112	985	900
9	4.4	10.4	114	1045	875
10	3.4	8.8	78	625	565
11	4.5	9.2	37	515	420
12	5.2	21.9	43	520	460

[a] Intensity of signal in arbitrary units. Values for vehicle were obtained from samples containing the complete enzymatic system (HRP/GSH or HRP/GSH/H_2O_2) without drug ($n = 1$). [b] Means of three values differing by less 2%; for vehicle, 100% means value of complete system without drug. [c] Peak potential (E_p) at pH 4.7 or 7.4 expressed in mV; means of three values differing by less 5 mV.

around 540 nm.[23] The absorbance of the chromogen MA/TBA is then measured to evaluate the degree of peroxidation (see Experimental Section). Different molecules such as the antioxidants are known to limit such lipid peroxidation.[24,25] Compound **1** was shown to react with such activated species and strongly reduced the absorbance in comparison with the control while compound **8** or **7** had weak effects.[26]

The sequence of reactivity from the most sensitive compound to oxidation to the least was as follows: **11** > **12** > **1** > **7** > **10** > **6** > **8** > **9** (Table 1).

Electrooxidation Potential. The voltammetric data were obtained using a carbon paste electrode. The pH value of 7.4 was chosen for physiological reasons, while the acetate buffer pH 4.7 was selected for its high buffering capacity, its weak interference with the electrochemical reaction, and also its allowing the detection of a cation radical. The latter can be evidenced by cyclic voltammetry, e.g., in the case of phenothiazines, which show a reversible one-electron redox pattern in aqueous acidic media.[27] All the investigated pyrido- and dibenzazepine compounds studied, except **16** and **17**, were irreversibly oxidized under the experimental conditions used. Compounds **16** and **17** showed a reversible redox process likely related to the iminoquinone/hydroxyclozapine redox structures.

The sequence of electrooxidation potential at pH 4.7 (OXID4.7) was as follows with potential range comprised between 450 and 1055 mV vs Ag/AgCl: **1** < **11** < **12** < **10** = **6** < **8** < **9** < **7** (Table 1). At pH 7.4, the potential values (OXID7.4) were found in the range of 375−975 mV. The sequence of electrooxidation potential at pH 7.4 was quite similar to that at pH 4.7 (Table 1).

Multivariate Analysis. Cluster analysis was used to establish correlations between the results obtained in these five experimental procedures. Figures 4−6 are so-called dendrograms (or taxonomic tree) obtained from hierarchical clustering analysis using Ward's method. It is a two-dimensional diagram illustrating the successive fusion of groups and subgroups of compounds until the stage is reached when all the compounds form a single cluster. Trifluoromethylsulfonyloxy and methoxy analogues may be classified into two clusters (Figure 4). The first cluster, clearly separated (level A) from the other one, includes compound **7**. The second one could be eventually separated in two groups (level B) which

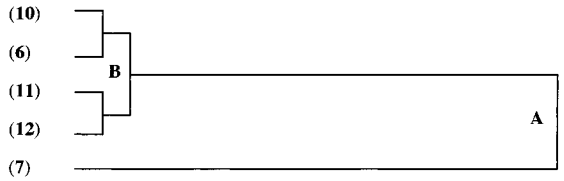

Figure 4. Dendrogram obtained for the methoxy and trifluoromethylsulfonyloxy analogues taking into account the values obtained for the five experimental variables (ESR1, ESR2, LIPO, OXID4.7, OXID7.4) and using a hierarchical cluster analysis model based on Ward's method and squared euclidean distances between measures.

contain methoxy (compounds **11** and **12**) and trifluoromethylsulfonyloxy (compounds **6** and **10**) derivatives, respectively.

By introducing data of methoxy and trifluoromethylsulfonyloxy analogues in the previously described model (Figure 5),[20] the newly investigated drugs were classified in different clusters (Figure 6). Compound **7** clustered separately in the group of potentially "safe" compounds (Figure 6, top), while other analogues (compounds **6** and **10**−**12**) were in the cluster of potentially "toxic" derivatives (Figure 6, bottom).

Discussion

Using the horseradish peroxidase-induced free radical model (ESR1), methoxy and trifluoromethylsulfonyloxy analogues presented low sensitivity to oxidation similar to **8** and **9**. In contrast to the pyridobenzazepine series, these original diazepine analogues were all less sensitive than the reference congener, clozapine (**1**). When adding H_2O_2 (ESR2 model), all compounds except **7** and **8** were oxidized (Table 1). This was also observed with pyridobenzazepine derivatives since oxazepine derivatives were insensitive to oxidation while diazepine and also thiazepine analogues enhanced the ESR signal.[20]

In lipid peroxidation conditions, an important difference was observed between diazepine analogues since methoxy derivatives (**11**, **12**) were readily oxidized (approximately 40% inhibition compared to the control which is equal to 100%) in comparison with trifluoromethylsulfonyloxy compounds (**6**, **7**, **10**) for which the values were found superior to 65% (Table 1).

Regarding the electrooxidation potential, diazepine analogues (**6**, **10**−**12**) like compound **1** possessed a low electrooxidation potential, while oxazepine **7** was oxidized at higher potential (> 900 mV) like **8** and **9** (Table 1).

A cluster analysis, taking into account the above five experimental parameters, was then used to classify trifluoromethylsulfonyloxy and methoxy analogues. This is a nonparametric method of multivariate statistics, whose purpose is to place objects into groups or clusters, not defined a priori. The objects in a given cluster tend to be similar and objects in different clusters dissimilar. Methoxy (**11**, **12**) and trifluoromethylsulfonyloxy (**6**, **10**) dibenzodiazepine analogues were classified by pairs (level B), but the distance coefficient (schematically represented by the length of the horizontal bar) between these pairs was very small (Figure 4). The oxazepine derivative **7** was found in a cluster clearly separated of the other one at level A (Figure 4).

Taking into account the five experimental variables in the hierarchical cluster analysis, the classification of

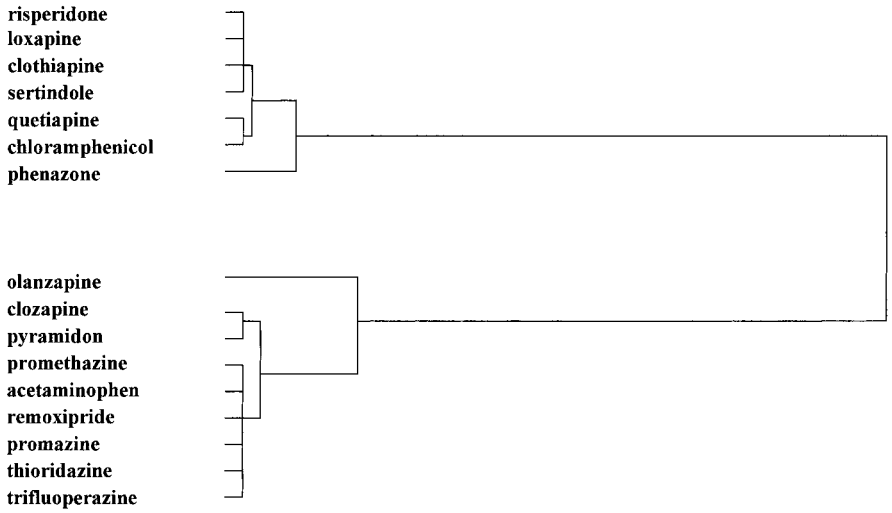

Figure 5. Dendrogram obtained for the reference compounds taking into account the values obtained for the five experimental variables (ESR1, ESR2, LIPO, OXID4.7, OXID7.4) and using a hierarchical cluster analysis model based on Ward's method and squared euclidean distances between measures (after Liégeois et al., 1999).[20]

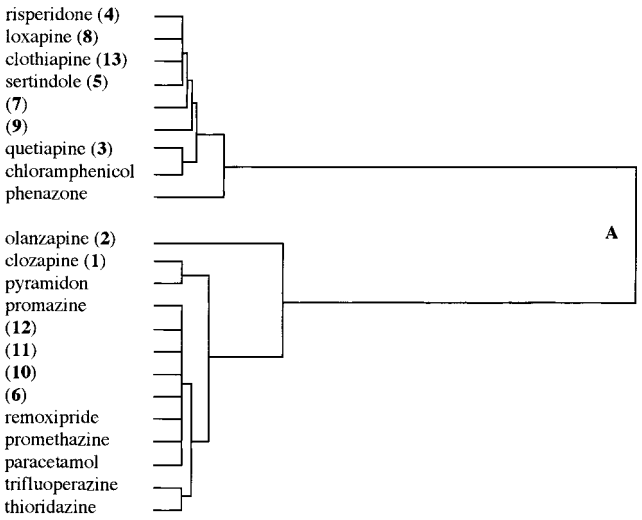

Figure 6. Dendrogram obtained when including methoxy analogues and trifluoromethylsulfonyloxy derivatives with the reference compounds taking into account the values obtained for the five experimental variables (ESR1, ESR2, LIPO, OXID4.7, OXID7.4) and using a hierarchical cluster analysis model based on Ward's method and squared euclidean distances between measures.

16 reference drugs according to their clinical hematological toxicity[11−15,28−34] or not[30,35−40] was 87.5% (14/16) correct (Figure 5). A clear separation was observed (level A). Clothiapine (**13**), loxapine (**8**), quetiapine (**3**), risperidone (**4**), and sertindole (**5**) were clustered in the cluster of "safe" compounds (Figure 5, top). A false classification was found for chloramphenicol and phenazone. The cluster of "unsafe" drugs (Figure 5, bottom) may be divided in three groups with no real false classification. For all of these compounds, differential incidence of hematological side effects is reported in the literature. In our mind, one parameter to take into account would be the duration of the treatment. However, determination of quantitative risk of the hematological problem is not easy to perform because the cellular and molecular mechanisms of such toxicity remain unknown and may be different depending on the cellular target.

By using the previously described multivariate analysis,[20] in order to determine an expected risk of toxicity of methoxy and trifluoromethylsulfonyloxy analogues in comparison with reference drugs, we found that compound **7** clustered among the "safe" drugs. Thus, the latter would be a good candidate for further development, similarly to **9**, in the context of our working hypothesis (Figure 6). The diazepine analogues (**6**, **10**−**12**) clustered together in the unsafe group. It is clear however that such in vitro screening would need further preclinical and/or clinical studies in order to confirm the safety of compound **7**.

Regarding the reference drugs (Figure 5), we found that the electrooxidation potential was the main parameter to classify compounds as potentially safe or unsafe.[20] In a relatively homogeneous series such as dibenzazepines, it could be possible to find some SORs according to the electrooxidation potential as is the case when SAR studies are determined on the basis of binding affinities. In this context, the electrooxidation potential of 21 diarylazepine analogues is reported in Table 2. Substitution by a hydroxy group, compound **6**, strongly decreases the electrooxidation potential compared to the chloro analogue (clozapine, **1**) which is generally the parent drug. In parallel to the *N*-demethylation and *N*-oxidation of the piperazine side chain,[41,42] metabolic pathways of clozapine (**1**) generate effectively some hydroxylated analogues. Then, such hydroxylated metabolites can be transformed in an iminoquinone species which can be readily trapped by various nucleophiles such as *N*-acetylcysteine or glutathione.[43] Thus, in a diazepine series, the hydroxy group (**16**, **17**) generates the most sensitive compound to oxidation followed by the chloro (clozapine, **1**) and methoxy (**11**), while the trifluoromethylsulfonyloxy group (**10**) tends to reduce the oxidation sensitivity (Table 2). This last substituent would apparently be interesting to be used as an electrophilic group with a stabilizing property. The position of the substituent in the tricyclic ring (2 or 8, after the dibenzazepine nomenclature in order to facilitate the discussion) had low influence on the oxidizability since a minimal variation in the different series examined was observed. Unsubstituted

Table 2. Electroooxidation Potential (E_p) of Diarylazepine Derivatives Measured at pH 7.4 Using a Carbon Paste Electrode

drug	lateral rings[a]	central ring[b]	substituent[c]	E_p[d]
1	DB	DIA	8-Cl	375
3	DB	THIA		800
6	DB	DIA	2-CF_3SO_2O	525
7	DB	OXA	2-CF_3SO_2O	975
8	DB	OXA	2-Cl	900
10	DB	DIA	8-CF_3SO_2O	565
11	DB	DIA	8-CH_3O	420
12	DB	DIA	2-CH_3O	460
13	DB	THIA	2-Cl	920
14	DB	THIA	8-Cl	950
15	DB	DIA	2-Cl	345
16	DB	DIA	8-OH	155
17	DB	DIA	8-OH	195
2	TB	DIA	2-CH_3[e]	150
9	PB	OXA	8-Cl	875
18	PB	DIA		465
19	PB	DIA	2-Cl	495
20	PB	DIA	2-CH_3	460
21	PB	DIA	8-CH_3	435
22	PB	THIA		880
23	PB	THIA	8-pyr-N[f]	915

[a] DB, dibenzo; PB, pyridobenzo; TB, thienobenzo. [b] DIA, diazepine; OXA, oxazepine; THIA, thiazepine. [c] Nomenclature of dibenzazepine analogues used for comparison. [d] Peak potential (E_p) at pH 4.7 or 7.4 expressed in mV; means of three values differing by less 5 mV. [e] Thienobenzazepine nomenclature. [f] 8-pyr-N, pyridine nitrogen atom in this position (see Figure 2).

analogues generally present a lower oxidation potential than the chloro analogues. Extending the number of investigated derivatives has permitted to show that the diazepine ring remained the most sensitive structure to oxidation compared to the oxazepine and thiazepine nuclei. The nature of the lateral rings had minimal influence on the oxidizability although pyridobenzazepine derivative **19** has an electrooxidation potential slightly higher than its dibenzazepine analogue, compound **15**.

To summarize, combining a hydroxy group with a diazepine nucleus provides the most sensitive compound with respect to oxidation, while the trifluoromethylsulfonyloxy group associated with an oxazepine or a thiazepine moiety would be the most stable analogue as in the case of compound **7** or **9**.

It is worthwhile to point out that our previous investigations have shown a possible relationship between the electrooxidation of dibenzazepine analogues and their pharmacological properties.[44] Voltammetric data have permitted to show that the electrooxidation occurred on the tricyclic ring and that the lone pair of electrons on the nitrogen (diazepine nucleus) is more readily available than the electron pairs of sulfur (thiazepine moiety) or oxygen (oxazepine moiety).[45] In the present work, although the detailed mechanism by which the electrooxidation of the investigated drugs occurs is currently unknown, the electrooxidation potential along with all the studied parameters can be regarded as valuable data in developing new tricyclic analogues with increased safety.

Experimental Section

Abbreviations: HRP, horseradish peroxidase; DTPA, diethylpentaacetic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide.

Drugs and Reagents. The following original dibenzazepine derivatives were evaluated: 8-(trifluoromethylsulfonyloxy)-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**10**), 8-methoxy-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]-diazepine (**11**), 2-methoxy-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**12**), 2-(trifluoromethylsulfonyloxy)-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**6**), and 2-(trifluoromethylsulfonyloxy)-11-(4-methyl-1-piperazinyl)-dibenz[*b*,*f*][1,4]oxazepine (**7**) (Figure 1). These drugs are obtained according to previously described methods.[8,9] HRP and GSH were obtained from Boehringer Mannheim; DTPA was from Sigma; hydrogen peroxide (H_2O_2) was from U.C.B. (Belgium); CHAPS was from Aldrich. Phosphate buffer (0.05 M $NaH_2PO_4 \cdot 2H_2O$ and Na_2HPO_4), TBA and TCA were obtained from Merck. Chelex 100 resin (200−400 mesh, sodium form) was from Biorad. DMPO was from Aldrich and purified with activated charcoal as previously described.[46] For ESR and lipid peroxidation experiments, drugs were dissolved in DMSO daily. All other chemical products were of analytical grade.

Biochemical Procedures. These methodologies are previously reported,[20] but in order to facilitate the reading, they are summarized below.

HRP-Catalyzed Oxidation (ESR1, ESR2). The first experiments were performed in 50 mM phosphate buffer (pH 7.5) containing 100 mM DMPO in a total volume of 1 mL. The reaction was started after addition of drug (final concentration 0.1 mM) to the complete system containing: HRP (25 mg/mL), GSH (10 mM), and DTPA (0.5 mM). This system (ESR1) was previously used.[16,19] In this study, we also used a modified peroxidation system (ESR2) by adding H_2O_2 at final concentration of 1 mM. In each case, the reaction mixture was then immediately transferred into a quartz flat cell in the ESR cavity. All measurements were recorded on a Bruker spectrometer ESP 300 E (Bruker Karlshure, Germany). ESR spectra were monitored at room temperature with following instrumental settings: microwave power 20 mW, modulation frequency 100 kHz, modulation amplitude 1.012 G, center of field 3480 ± 50 G and sweep width 100 G, number of scans 3. Classically, each drug is tested one time. A clozapine and a blank control are used each experimental day in order to check the response of the biochemical system.

Lipid Peroxidation Experiments (LIPO). The stock solutions of drugs (0.01 M), prepared in DMSO, were diluted at a final concentration of 0.1 mM in phosphate buffer pH 7.4 (total volume 2 mL). To a mixture containing: 200 mg of CHAPS, 0.2 mL of linoleic acid (final concentration 6.4 mM) and 100 mL of chelexed phosphate buffer was added 20 μL of 0.01 M drug. The mixture reaction was γ-irradiated (from Cesium-137 source at the rate of 10 000 rads). Then, 0.5 mL of the irradiated solution was added to 0.5 mL of TCA and 2 mL of TBA (26 mM) in 50 mM of Tris-HCl pH 7. Each assay was done in triplicate. After extraction with 2 mL of *n*-butanol, the absorbance of each sample was measured by monitoring changes by using the thiobarbituric method.[23] The absorbance was measured at 540 nm with *n*-butanol as internal reference on Perkin-Elmer Lambda 15 UV:VIS spectrophotometer.

Electrooxidation Potential Measurements (OXID4.7, OXID7.4). Voltammetric measurements have been realized using a CV 27 voltammograph (BAS West Lafayette) connected to a Hewlett-Packard 7090Ax-y recorder. The experiments were performed in a three-electrode cell containing the working electrode, a platinum auxiliary electrode, and a Ag/AgCl in 3 M KCl reference electrode. The working electrode was made of carbon paste (solid paraffin/graphite 34/66, w/w). Before each voltammogram, the electrode surface was smoothed on a soft paper. The molecules were first dissolved in methanol. The investigated solutions contained the drug (0.1 mM) in the buffer with 2% methanol. Linear scan voltammograms were recorded in 0.1 M phosphate buffer pH 7.4 at a scan rate of 25 mV/s and at room temperature (OXID7.4). Similar measurements were made at pH 4.7 in 0.25 M acetate buffer (OXID4.7). Each measure was made in triplicate and the

variations were less than 5 mV. The oxidizability of the compounds was estimated on the basis of their peak potential (E_p).

Statistical Analysis. Hierarchical cluster analyses using Ward's method and squared euclidean distances between measures were used to identify homogeneous groups of reference drugs based on each variable taken separately (peroxidase-catalyzed oxidation with HRP/GSH or HRP/H_2O_2/GSH, lipid peroxidation, oxidation potential at pH 4.7 and 7.4) and based on all variables taken together (MVA). The percentage of correct classification of safe/unsafe drugs was calculated in each case. The same analysis was replicated after adding the original dibenzazepine derivatives to the reference compounds.

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