



**HAL**  
open science

# **Bacterial Plate Assays and Electrochemical Methods: An Efficient Tandem for Evaluating the Ability of Catechol–Thioether Metabolites of MDMA (“Ecstasy”) to Induce Toxic Effects through Redox-Cycling**

Anne Felim, Amparo Urios, Anne Neudörffer, Guadalupe Herrera, Manuel Blanco,  
Martine Largeron

## ► To cite this version:

Anne Felim, Amparo Urios, Anne Neudörffer, Guadalupe Herrera, Manuel Blanco, et al.. Bacterial Plate Assays and Electrochemical Methods: An Efficient Tandem for Evaluating the Ability of Catechol–Thioether Metabolites of MDMA (“Ecstasy”) to Induce Toxic Effects through Redox-Cycling. *Chemical Research in Toxicology*, 2007, 20 (4), pp.685-693. <10.1021/tx6003584>. <hal-02384959>

**HAL Id: hal-02384959**

**<https://hal.science/hal-02384959v1>**

Submitted on 11 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

# Bacterial Plate Assays and Electrochemical Methods: an Efficient Tandem for Evaluating the Ability of Catechol-thioether Metabolites of MDMA (“Ecstasy”) to Induce Toxic Effects through Redox-cycling

Anne Felim,<sup>†</sup> Amparo Urios,<sup>‡</sup> Anne Neudörffer,<sup>†</sup> Guadalupe Herrera<sup>‡</sup>, Manuel Blanco,<sup>‡</sup> and Martine  
Largeron<sup>\*,†</sup>

*<sup>†</sup>UMR 8638 CNRS - Université Paris Descartes, Synthèse et Structure de Molécules d'Intérêt  
Pharmacologique, Faculté des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire,  
75270 Paris cedex 06, France and <sup>‡</sup>Centro de Investigacion Principe Felipe, Avenida Autopista del  
Saler 16, 46013 Valencia, Spain*

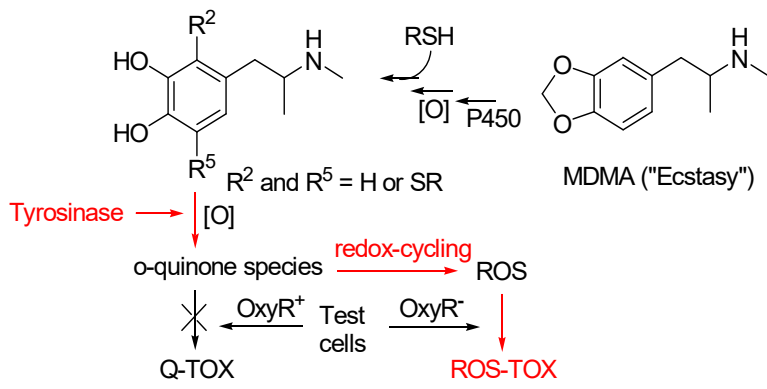
## RECEIVED DATE

TITLE RUNNING HEAD: Redox Toxicity of Catechol-thioether Metabolites of MDMA

CORRESPONDING AUTHOR FOOTNOTE: 33 01 53 73 96 46; fax: 33 01 44 07 35 88;

E-mail: [martine.largeron@univ-paris5.fr](mailto:martine.largeron@univ-paris5.fr) (M.L.)

## Table of Contents Graphic



**Abstract.** Several catechol-thioether metabolites of MDMA (ecstasy), three mono-adducts, 5-(glutathion-S-yl)-N-methyl- $\alpha$ -methyldopamine **1**, 5-(N-acetylcystein-S-yl)-N-methyl- $\alpha$ -methyldopamine **2**, and 5-(cystein-S-yl)-N-methyl- $\alpha$ -methyldopamine **3**, and two bi-adducts, 2,5-bis(glutathion-S-yl)-N-methyl- $\alpha$ -methyldopamine **4** and 2,5-bis(N-acetylcystein-S-yl)-N-methyl- $\alpha$ -methyldopamine **5**, have been synthesized through an environmentally friendly one-pot electrochemical procedure. Their cytotoxicity profiles were further characterized using simple *Escherichia coli* plate assays, and compared with those of N-methyl- $\alpha$ -methyldopamine (HHMA), dopamine (DA) and its corresponding catechol-thioether conjugates ( mono-adducts **6-8** and bi-adducts **9, 10**). Toxicity mediated by reactive oxygen species (ROS-TOX) was detected in the OxyR<sup>-</sup> assay, using cells sensitive to oxidative stress due to a deficiency in the OxyR protein. Toxicity arising from the high susceptibility of quinone toward endogenous nucleophiles (Q-TOX) was detected using OxyR<sup>+</sup> cells, in the presence of tyrosinase to promote the catechol oxidation to the corresponding *o*-quinone. At the exclusion of 5-(cystein-S-yl) mono-conjugate **3**, which was devoid of any toxicity, all compounds produced ROS-TOX, which was enhanced in the presence of tyrosinase, suggesting that the generated *o*-quinone (or *o*-quinone-thioether) species can enter redox cycles through its semiquinone radical leading to the formation of ROS. The sequence order of toxicity was: HHMA  $\cong$  **1**  $\cong$  **2**  $\cong$  **5**  $\gg$  **7**  $>$  DA  $\cong$  **4**  $>$  **10**  $>$  **6**  $>$  **8**. In contrast, no Q-TOX arising from binding of quinones with cellular nucleophiles was evidenced, even in the presence of tyrosinase. Finally, taking into account that several different pathways could contribute to the overall MDMA toxicity, and that HHMA and catechol-thioether conjugates **1-5** have not been undoubtedly established as *in vivo* toxic metabolites of MDMA, it can be suggested that these compounds could participate in the toxic effects of this drug through the efficiency of redox active quinonoid centers generating ROS.

## Introduction

(±)-3,4-methylenedioxymethamphetamine (MDMA, also known as “ecstasy”) is a synthetic psychoactive drug possessing stimulant and hallucinogenic properties. The recreational use of MDMA is prevalent, mainly among young people, despite warnings of irreversible damage to the central nervous system (1-6). Furthermore, MDMA consumption produces a variety of systemic and organ-specific adverse effects (7-9). Consequently, MDMA abuse has the potential to give rise to a major public health problem.

There is a general consensus that MDMA metabolism, resulting in the formation of highly redox active metabolites, is required for the expression of MDMA-induced neurotoxicity (10-18), hepatotoxicity (19, 20), nephrotoxicity (21) and cardiotoxicity (22). However, the mechanism by which these metabolites initiate and propagate the toxic events remains to be thoroughly clarified.

In humans, MDMA is essentially metabolized, via *o*-demethylenation, into 3,4-dihydroxymethamphetamine (HHMA), while N-demethylation into 3,4-methylenedioxyamphetamine (MDA) constitutes a minor metabolic pathway (Scheme 1) (23). Because of its catechol moiety, HHMA can be easily oxidized to the corresponding *o*-quinone species Q<sub>1</sub>, which can enter redox cycles through its semiquinone radical SQ<sub>1</sub><sup>•</sup>, leading to the formation of reactive oxygen species (ROS) including superoxide, hydrogen peroxide and, ultimately, the hydroxyl radical. The *o*-quinone intermediate is also a Michael acceptor, which can be conjugated with thiol nucleophiles such as GSH, to form 5-glutathion-S-yl-N-methyl- $\alpha$ -methyldopamine. This GSH conjugate remains redox active, being readily oxidized to the quinone-thioether species Q<sub>2</sub>, which, after the reductive addition of a second molecule of GSH, yields the 2,5-bis-glutathion-S-yl conjugate, also oxidizable to the quinone-thioether species Q<sub>3</sub> (Scheme 1).

Taking all together, the ability of HHMA and its catechol-thioether conjugates to undergo redox cycling and produce ROS, might provide a rationale for the role of these metabolites in MDMA-induced toxicity (15,24,25). This prompted us, first, to develop a straightforward one-pot electrochemical

procedure allowing the synthesis of several catechol-thioether metabolites of MDMA **1-5** (Figure 1) and, second, to evaluate their cytotoxicity profiles through simple *Escherichia coli* plate assays, which allowed the discrimination of toxicity mediated by ROS (ROS-TOX) from that induced by the reaction of *o*-quinones with cellular nucleophiles (Q-TOX) (26).

We have recently shown that OxyR<sup>-</sup> cells, deficient in the OxyR function, are adequate targets for the detection of ROS-TOX, a toxicity sensitive to inhibition by antioxidant defenses (27-29). The OxyR protein is a redox-sensitive transcriptional regulator, that mediates the induction of genes encoding antioxidant enzymes such as catalase and peroxiredoxin alkyhydroperoxide reductase (30,31). Consequently, this induction is prevented in OxyR protein-deficient cells.

Toxicity arising from binding of quinones with cellular nucleophiles (Q-TOX) is not inhibited by antioxidant enzymes and can be detected in OxyR<sup>+</sup> cells (28). Detection of Q-TOX has been improved by adding to the assay nitric oxide, which promoted quinol oxidation (28,29). In the present work, the two-electron oxidation of quinols by tyrosinase was used to promote quinone formation.

At this point, it should be noted that catechol-thioether metabolites of MDMA are known to stimulate dopamine transport into cells that express the human serotonin transporter, and hence, can potentiate ROS generation through dopamine deamination and/or autoxidation (25). So, for comparison, the same bacterial plate assays have also been applied to dopamine (DA) and its related catechol-thioether conjugates **6-10** (Figure 1).

In this paper, we demonstrate that HHMA, and most of its catechol-thioether conjugates **1-5**, behave as potent inducers of toxicity mediated by ROS, with a greater efficiency than DA and its related thioether conjugates **6-10**. In addition, the results suggest an important role of the *o*-quinone species as redox active centers generating ROS.

## Experimental Procedures

**Chemistry.** All reagents and solvents (HPLC grade) were commercial products of the highest available purity and were used as supplied. Dopamine hydrochloride (DA, HCl) was purchased from Aldrich. (±)-*N*-methyl- $\alpha$ -methyl-dopamine hydrobromide (*N*-Me- $\alpha$ -Me-DA, HBr) was synthesized in four steps from commercially available 3,4-dimethoxy-benzaldehyde and nitroethane through procedures previously reported (32-35).

HPLC was carried out using a Waters system consisting of a 600E multisolvent delivery system, a Rheodyne-type loop injector, and a 2487 dual-channels UV-visible detector set at 254 and 278 nm. A mixture of three solvents constituted the mobile phase: acetonitrile (MeCN), solvents A and B. Solvent A was prepared by adding 1‰ concentrated trifluoroacetic acid (TFA) to deionized water. Solvent B was prepared by adding 0.5‰ TFA to a mixture of equivalent volumes of deionized water and methanol. Semi-preparative reversed-phase HPLC was performed using a 250 x 20 mm – 5  $\mu$ m-Kromasil C18 column and a 2 mL loop injector, while, for analytical reversed-phase HPLC, a 250 x 4.6 mm - 5  $\mu$ m- Kromasil C18 column, together with a 50  $\mu$ L loop injector, were used.

$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were performed on a Bruker AC-300 spectrometer operating at 300 and 75 MHz, respectively. Chemical shifts are expressed as  $\delta$  units (part per million) downfield from TMS (tetramethylsilane). The measurements were carried out using the standard pulse-sequences. The carbon type (methyl, methylene, methine or quaternary) was determined by DEPT experiments.  $^1\text{H}$  NMR spectra of all catechol-thioether conjugates **1-10** are included in the supporting information as a proof of their identity.

**Electrochemistry.** Electrochemical measurements were made with a Radiometer-Tacussel PRG 5 multipurpose polarograph, which was used only as a rapid-response potentiostat. For cyclic voltammetry, triangular waveforms were supplied by a Tacussel GSTP 4 function generator. Current-potential curves were recorded on a Schlumberger SI 8312 instrument. The cell was a Radiometer-Tacussel CPRA water-jacketed cell working at a temperature of 25°C. The working electrode was a

platinum disk, carefully polished before each voltammogram with an aqueous alumina suspension. The counter electrode was a platinum electrode Tacussel Pt 11. The reference electrode was an Ag/AgCl electrode, to which all potentials quoted are referred.

Controlled-potential electrolyses were carried out using a DEA 332 electrochemical analyser (Radiometer, Copenhagen), in a cylindrical three-electrode divided cell (9 cm diameter). In the main compartment, a cylindrical platinum grid (area = 60 cm<sup>2</sup>) served as the anode (working electrode). A platinum sheet was placed in the concentric cathodic compartment (counter-electrode), which was separated from the main compartment with a glass frit. The reference electrode was as above described.

**5-(glutathion-S-yl)-N-methyl- $\alpha$ -methyl-dopamine (1).** *General procedure A:* A solution of ( $\pm$ )-N-methyl- $\alpha$ -methyl-dopamine hydrobromide (N-Me- $\alpha$ -Me-DA, HBr) (65.5 mg, 0.25 mmol), in 0.2 M HCl (250 mL), was oxidized under nitrogen, at room temperature, at a platinum grid whose potential was fixed at + 1.0 V versus Ag/AgCl. After the consumption of 2 electrons per molecule, 2 equiv of glutathione (157 mg, 0.50 mmol) were added to the pale yellow solution which turned colorless. The reaction mixture was immediately frozen at – 80°C and then freeze-dried. The residue was subdivided in fractions of about 25 mg. Each fraction was dissolved in 2 mL of water, and then purified by semi-preparative reversed-phase HPLC, using a mixture of solvent A/solvent B 70/30 as the eluent (flow rate: 3 mL min<sup>-1</sup>). Fractions containing 5-(glutathion-S-yl)-N-Me- $\alpha$ -Me-DA **1** were collected, immediately frozen at –80°C, and then freeze-dried. Compound **1** was isolated as a white solid (78 mg, 0.16 mmol), in 64% yield. Its degree of purity (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 94/6 – flow rate: 1 mL min<sup>-1</sup>). <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  1.12 (d,  $J$  = 6.5 Hz, 3H), 2.01 (m, 2H), 2.36 (t,  $J$  = 7.5 Hz, 2H), 2.56 (s, 3H), 2.62 (m, 1H), 2.78 (m, 1H), 3.06 (m, 1H), 3.26 (m, 2H), 3.70 (s, 2H), 3.81 (m, 1H), 4.28 (m, 1H), 6.66 (s, 1H), 6.75 (s, 1H). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  15.5, 26.6, 30.6, 31.9, 35.4, 38.6, 42.1, 53.8, 54.4, 57.0, 118.0, 120.1, 126.8, 129.0, 144.7, 145.2, 172.9, 174.0, 175.2 ( $\times$  2). MS (ES<sup>+</sup>),  $m/z$  487 [M+H]<sup>+</sup>.

**5-(N-acetylcystein-S-yl)-N-methyl- $\alpha$ -methyl-dopamine (2).** The general procedure A, replacing glutathione by N-acetylcysteine (83 mg, 0.50 mmol), afforded, after semi-preparative reversed-phase

HPLC (eluent: solvent A/ solvent B 80/20 - flow rate: 16.5 mL min<sup>-1</sup>), 5-(*N*-acetylcystein-S-yl)-*N*-Me- $\alpha$ -Me-DA **2** as a white solid (56 mg, 0.165 mmol), in 66% yield. Its degree of purity (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 93/7 – flow rate: 1 mL min<sup>-1</sup>). <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  1.10 (d, *J* = 6.5 Hz, 3H), 1.79 (s, 3H), 2.55 (s, 3H), 2.60 (m, 1H), 2.77 (m, 1H), 3.05 (m, 1H), 3.27 (m, 2H), 4.26 (m, 1H), 6.64 (s, 1H), 6.73 (s, 1H). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  15.4, 22.2, 30.5, 35.2, 38.5, 53.1, 56.9, 117.8, 120.1, 126.8, 128.8, 144.6, 145.1, 174.4 ( $\times$  2). MS (ES<sup>+</sup>), *m/z* 343 [M+H]<sup>+</sup>.

**5-(cystein-S-yl)-*N*-methyl- $\alpha$ -methyl-dopamine (3).** The general procedure A, replacing glutathione by cysteine (62 mg, 0.50 mmol), afforded, after semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 90/10 - flow rate: 11.5 mL min<sup>-1</sup>), 5-(cystein-S-yl)-*N*-Me- $\alpha$ -Me-DA **3** as a white solid (38 mg, 0.13 mmol), in 50% yield. Its degree of purity (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 97/3 – flow rate: 0.8 mL min<sup>-1</sup>). <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  1.14 (d, *J* = 6.5 Hz, 3H), 2.57 (s, 3H), 2.65 (m, 1H), 2.80 (m, 1H), 3.33 (m, 3H), 3.86 (m, 1H), 6.71 (s, 1H), 6.85 (s, 1H). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  15.5, 30.6, 35.1, 38.6, 53.4, 56.9, 118.4, 119.3, 127.3, 129.1, 145.1, 145.4, 171.6. MS (ES<sup>+</sup>), *m/z* 301 [M+H]<sup>+</sup>.

**2,5-bis-(glutathion-S-yl)-*N*-methyl- $\alpha$ -methyl-dopamine (4).** *General procedure B:* A solution of *N*-Me- $\alpha$ -Me-DA, HBr (65.5 mg, 0.25 mmol) in 0.2 M hydrochloric acid (250 mL), was oxidized under nitrogen, at room temperature, at a platinum grid (*E* = + 1.0 V versus Ag/AgCl). After the consumption of 2 electrons per molecule, the electrolysis was stopped and 3 equiv of glutathione (235 mg, 0.75 mmol) were added. When the yellow electrolysis solution became colorless, it was oxidized again at + 1.0 V versus Ag/AgCl. After exhaustive electrolysis (*n* = 5.8, that is 2.0 for the first step and 3.8 for the second one), 2 equiv of glutathione (157 mg, 0.50 mmol) were added to the reaction mixture, which was immediately frozen at – 80°C and then freeze-dried. The residue was subdivided in fractions of about 25 mg. Each fraction was dissolved in 2 mL of water and then purified by semi-preparative reversed-phase HPLC, using a mixture of solvent A/ solvent B 72/28 as the eluent (flow rate: 3 mL min<sup>-1</sup>). Fractions containing 2,5-bis-(glutathion-S-yl)-*N*-Me- $\alpha$ -Me-DA (**4**) and 5-(glutathion-S-yl)-*N*-Me- $\alpha$ -Me-DA (**1**)

were collected individually, immediately frozen at  $-80^{\circ}\text{C}$  and then freeze-dried. Compound **4** was isolated as the major product in 50% yield (pale pink solid, 99 mg, 0.13 mmol), along with compound **1** as the minor one, in 22% yield (26.5 mg, 0.055 mmol). The degree of purity for compound **4** (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 94/6 – flow rate:  $0.8\text{ mL min}^{-1}$ ).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.09 (d,  $J = 6.3\text{ Hz}$ , 3H), 2.03 (m, 4H), 2.36 (m, 4H), 2.60 (s, 3H), 2.85 (m, 1H), 3.11 and 3.31 (m, 6H), 3.72 (s, 2H), 3.75 (s, 2H), 3.83 (m, 2H), 4.25 (m, 2H), 6.85 (s, 1H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  14.7, 25.4, 25.5, 30.0, 30.9 ( $\times 2$ ), 34.6, 35.0, 36.9, 37.1, 40.1, 52.4 ( $\times 2$ ), 52.6, 53.4, 56.2, 118.9, 120.9, 125.8, 131.8, 143.8, 146.7, 171.9 ( $\times 2$ ), 172.1 ( $\times 2$ ), 172.7 ( $\times 2$ ), 174.1 ( $\times 2$ ). MS (ES+),  $m/z$  792  $[\text{M}+\text{H}]^+$ .

**2,5-bis-(*N*-acetylcystein-S-yl)-*N*-methyl- $\alpha$ -methyl-dopamine (5).** The general procedure B, replacing glutathione by *N*-acetylcysteine (208 mg, 1.25 mmol, overall quantity), afforded, after exhaustive electrolysis ( $n = 5.5$ , that is 2 for the first step and 3.5 for the second one) and semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 93/17 - flow rate:  $14\text{ mL min}^{-1}$ ), 2,5-bis-(*N*-acetylcystein-S-yl)-*N*-Me- $\alpha$ -Me-DA **5** in 44% yield (white solid, 55 mg, 0.11 mmol), along with compound **2** in 22% yield (19 mg, 0.055 mmol). The degree of purity for compound **5** (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 92/8 – flow rate:  $0.8\text{ mL min}^{-1}$ ).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  1.08 (d,  $J = 6.5\text{ Hz}$ , 3H), 1.75 (d,  $J = 3.5\text{ Hz}$ , 3H), 1.80 (d,  $J = 4.5\text{ Hz}$ , 3H), 2.59 (s, 3H), 2.80 (m, 1H), 3.09 and 3.25 (m, 6H), 4.25 (m, 2H), 6.84 (s, 1H).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  14.8, 21.5( $\times 2$ ), 30.0, 34.5, 34.7, 37.0, 52.6, 53.5, 56.2, 119.4, 121.0, 126.1, 131.6, 143.9, 146.6, 173.8 ( $\times 4$ ). MS (ES+),  $m/z$ : 504  $[\text{M}+\text{H}]^+$ , 526  $[\text{M}+\text{Na}]^+$ .

**5-(glutathion-S-yl)-dopamine (6).** The general procedure A, replacing ( $\pm$ )-*N*-methyl- $\alpha$ -methyl-dopamine hydrobromide by dopamine hydrochloride (DA, HCl) (47.5 mg, 0.25 mmol) afforded, after semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 95/5 - flow rate:  $11.5\text{ mL min}^{-1}$ ), 5-(glutathion-S-yl)-DA **6** as a white solid (74.5 mg, 0.16 mmol) in 65% yield. Its degree of purity (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 97/3 – flow rate:  $1.0\text{ mL min}^{-1}$ ). Spectroscopic data were identical to those previously reported (36-38).

**5-(*N*-acetylcystein-S-yl)-dopamine (7).** The general procedure A, replacing *N*-methyl- $\alpha$ -methyl-dopamine hydrobromide by dopamine hydrochloride (DA, HCl) (47.5 mg, 0.25 mmol), and glutathione by *N*-acetylcysteine (83 mg, 0.50 mmol), afforded, after semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 88/12 - flow rate: 11 mL min<sup>-1</sup>), 5-(*N*-acetylcystein-S-yl)-DA **7** as a white solid (43 mg, 0.137 mmol), in 55% yield. Its degree of purity (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 94/6 – flow rate: 0.8 mL min<sup>-1</sup>). Spectroscopic data were identical to those previously reported (38,40).

**5-(cystein-S-yl)-dopamine (8).** The general procedure A, replacing ( $\pm$ )-*N*-methyl- $\alpha$ -methyl-dopamine hydrobromide by dopamine hydrochloride (DA, HCl) (47.5 mg, 0.25 mmol), and glutathione by cysteine (62 mg, 0.50 mmol), afforded, after semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 88/12 - flow rate: 3 mL min<sup>-1</sup>), 5-(cystein-S-yl)-DA **8** as a white solid (43 mg, 0.16 mmol) in 63 % yield. Its degree of purity (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 95/5 – flow rate: 0.6 mL min<sup>-1</sup>). Except for <sup>13</sup>C NMR spectrum which was not described, spectroscopic data were identical to those previously reported (41). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  31.8, 34.5, 40.4, 53.0, 117.2, 118.7, 126.1, 129.3, 144.2, 144.7, 171.6.

**2,5-bis-(glutathion-S-yl)-dopamine (9).** The general procedure B, replacing ( $\pm$ )-*N*-methyl- $\alpha$ -methyl-dopamine hydrobromide by dopamine hydrochloride (DA, HCl) (47.5 mg, 0.25 mmol) afforded, after exhaustive electrolysis (n = 5, that is 2 for the first step and 3 for the second one) and semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 78/22 - flow rate: 2.4 mL min<sup>-1</sup>), 2,5-(glutathion-S-yl)-DA **9** as a white solid (122 mg, 0.16 mmol) in 64% yield, along with compound **6** as the minor one, in 24% yield (28 mg, 0.06 mmol). The degree of purity for compound **9** (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 94/6 – flow rate: 0.7 mL min<sup>-1</sup>). Except for <sup>13</sup>C NMR spectrum which was not described, spectroscopic data were identical to those previously reported (36,37). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  26.4, 26.5, 31.8 ( $\times$  2), 32.0, 35.2, 35.8, 41.1, 41.9 ( $\times$  2), 53.6 ( $\times$  2), 54.1 ( $\times$  2), 119.5, 121.9, 126.0, 133.5, 144.4, 147.5, 172.9 ( $\times$  2), 173.7 ( $\times$  2), 175.1 ( $\times$  4).

**2,5-bis-(*N*-acetylcystein-S-yl)-dopamine (10).** The general procedure B, replacing ( $\pm$ )-*N*-methyl- $\alpha$ -methyl-dopamine hydrobromide by dopamine hydrochloride (DA, HCl) (47.5 mg, 0.25 mmol) and glutathione by *N*-acetylcysteine (208 mg, 1.25 mmol, overall quantity), afforded, after exhaustive electrolysis ( $n = 5.5$ , that is 2 for the first step and 3.5 for the second one) and semi-preparative reversed-phase HPLC (eluent: solvent A/ solvent B 80/20 - flow rate: 6 mL min<sup>-1</sup>), 2,5-bis-(*N*-acetylcystein-S-yl)-DA **10** in 62% yield (white solid, 74 mg, 0.16 mmol), along with compound **7** in 18% yield (14.2 mg, 0.045 mmol). The degree of purity for compound **10** (99%) was determined by analytical HPLC (eluent: solvent A/ MeCN 91/9 – flow rate: 0.9 mL min<sup>-1</sup>). Except for <sup>13</sup>C NMR spectrum which was not described, spectroscopic data were identical to those previously reported (39,40). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  21.5 ( $\times 2$ ), 31.2, 34.5, 34.8, 40.3, 52.8, 53.6, 119.1, 121.2, 125.6, 132.5, 143.7, 146.7, 173.9, 174.0.

**Bacterial plate cytotoxicity assays.** The *E. coli* strains were IC5282 (a *trp*<sup>+</sup> derivative of strain WP2 *uvrA trpE65/pKM101*), IC5204 (as IC5282 but carrying the  $\Delta$ *oxyR30* mutation (42)), and IC2880 (a *trp::Tn10* derivative of strain WP2 *uvrA trpE65*). Cultures were prepared by inoculating 100  $\mu$ L from frozen permanents into 10 mL of nutrient broth (Oxoid Nutrient Broth No. 2), and incubating overnight at 37°C. Solid minimal E4 medium contained 15 g Difco agar and 4 g glucose per liter of Vogel-Bonner E buffer (containing MgSO<sub>4</sub> · 7 H<sub>2</sub>O (10 g), citric acid monohydrate (100 g), K<sub>2</sub>HPO<sub>4</sub> (500 g) and NaH<sub>2</sub>NH<sub>4</sub>PO<sub>4</sub> · 7 H<sub>2</sub>O (175 g) per liter of distilled water). Top agar contained 6 g Difco agar and 5 g NaCl per liter of distilled water. In the assays, test cells (from IC5282 and IC5204 strains) were exposed to the compound in the presence of *trp*<sup>-</sup> filler cells (from IC2880 strain), unable to growth in the minimal E4 medium, which was used to obtain a background lawn. To 100  $\mu$ L of the bacterial mix, containing approximately 800 test cells (from a dilution, in E buffer, of an overnight broth culture) and 3x10<sup>7</sup> filler cells (from an undiluted overnight broth culture) were added: 100  $\mu$ L of a suitable dilution of the test compound, and 2.5 mL of molten top agar. Where indicated, mushroom tyrosinase (EC 1.14.18.1; Sigma) was added to the assay (100  $\mu$ L of a suitable dilution from a starting solution in 0.1 M sodium phosphate pH 7.0). The mixture was poured on plates containing solid E4 medium (about 35

mL), which were incubated at 37°C, for 24 or 48 h. % Survival = [colony forming units (CFU) per plate at each dose divided by CFU per plate at zero dose] x 100. Data were the mean of at least three experiments. Error bars represent SD and are shown when larger than 10%. From the dose-response curves obtained in the assays, a parameter indicating the cytotoxic potency, the TC<sub>50</sub> value, was calculated as the concentration (μmol/plate) needed to reduce the survival to 50%.

## Results and Discussion

**Chemistry. Synthesis of catechol-thioether mono-adducts 1-3.** A simple method for synthesizing a catechol-thioether mono-adduct involves the oxidation of the catechol with mushroom tyrosinase, in the presence of a thiol. The reaction proceeds via formation of the *o*-quinone intermediate (43), followed by 1,6-Michael addition reaction of thiol. This method has been recently used for the synthesis of the catechol-thioether metabolite **2** using *N*-acetylcysteine as the thiol, and HHMA as the starting catechol (yield not given) (17). To the best of our knowledge, the syntheses of catechol-thioether mono-adducts **1** and **3** have never been reported, although similar conjugates, isolated from the enzymatic oxidation of 3,4-dihydroxyamphetamine (10,18), dopamine or dopa (36,37,44,45), are known. However, the enzymatic procedure is not adapted for routine syntheses, because it is too expensive and not suitable to yield substantial amounts of catechol-thioether mono-conjugates.

When applied to HHMA, existing chemical methods reported for the synthesis of catechol thioether mono-conjugates of dopamine or dopa, which use diverse oxidizing agents such as hydrobromic acid, silver oxide and ceric ammonium nitrate (46-48), only gave low yields of the corresponding conjugates. Furthermore, the workup and isolation procedures were not suitable for the synthesis of metabolites in high purity grade (99%) as required for biological evaluation, because the products were separated by column chromatography on Dowex using HCl as the eluent.

For a long time, electrochemical oxidation has proved to be an efficient tool for the generation of highly electrophilic quinones species, which can be further scavenged by diverse entities such as amines (49,50), enamines (51) or thiols. Accordingly, unstable *o*-quinone species have been electrogenerated from dopamine (36, 39-41), norepinephrine (52) or *N*-acetyldopamine (53), and then scavenged by thiol residues to afford the corresponding catechol-thioether adducts. Consequently, we turned our attention toward these methods for producing the catechol-thioether conjugates **1-3**.

The mono-adducts **1-3** have been prepared following a procedure close to that previously reported in the case of dopamine (36, 39-41), with some modifications (54). We found that the anodic oxidation of

HHMA had to be conducted in the absence of thiol substrate, because the catechol-thioether mono-adduct was also electroactive at the applied potential, giving unwanted additional oxidation products.

The cyclic voltammogram of HHMA (1 mM), in deaerated 0.2 mM phosphate buffered aqueous solution (pH 7.3), at room temperature, showed, at a platinum anode, an oxidation peak Pa at + 200 mV versus Ag/AgCl, due to a two-electron process, the sweep rate being 200 mV s<sup>-1</sup> (Figure 2a). On the reverse sweep, the lack of peak Pc illustrated the irreversibility of the overall two-electron oxidation process, indicating that the electrogenerated *o*-quinone species was reactive enough to be engaged in a fast follow-up chemical reaction. As previously described in the case of dopamine (41), the putative *o*-quinone species underwent rapid intramolecular cyclization, leading to a redox active indoline derivative (as shown by the presence of the redox system (Pa',Pc') in figure 2a) which, after subsequent oxidation reaction, rapidly afforded insoluble melanin-like polymers. Consequently, to circumvent this problem, the anodic electrolysis of HHMA was performed under acidic conditions. Then, the protonation of the amino group prevented the nucleophilic attack at the origin of the formation of the indoline species.

Accordingly, the cyclic voltammogram of HHMA (1 mM), in deaerated 0.2M HCl aqueous solution, at room temperature, showed, at a platinum anode, an oxidation peak Pa at + 560 mV versus Ag/AgCl, due to a two-electron process, the sweep rate being 10 mV s<sup>-1</sup>. As can be seen in Figure 2b, a cathodic peak Pc appeared on the reverse sweep at + 470 mV versus Ag/AgCl, illustrating the quasireversibility of the two-electron transfer that could be assigned to the *o*-quinol/*o*-quinone redox couple.

When the controlled potential of the platinum anode was fixed at + 1.0 V versus Ag/AgCl, which is at a potential following the anodic peak observed in cyclic voltammetry, a coulometric value of  $2.0 \pm 0.1$  was found for the number of electrons (n) involved in the oxidation of one molecule of HHMA into the transient *o*-quinone species. The latter was rather stable under the aforementioned experimental conditions, indicating that the combination of the acidic aqueous conditions, together with the low concentration of HHMA (1mM), prevented both cyclization to indoline derivatives and polymerization side-reactions. Subsequent addition of 2 equiv of thiol derivative resulted in the immediate discoloration of the yellow solution, due to the formation of the catechol-thioether conjugate. Treatment of the

electrolysis solution afforded, after semi-preparative reversed-phase HPLC (See Experimental Section), catechol-thioether mono-adducts **1-3**, in yields ranging from 50 to 66% (Table 1).

**Synthesis of catechol-thioether bi-adducts 4 and 5.** The synthesis of bis-conjugates, resulting from two consecutive 1,6-addition reaction of thiol, has received only scant attention, most of the previous works being restricted to the synthesis of catechol-thioether mono-adducts (36-41). The corresponding bis-catechol-thioether conjugate was then isolated as a by-product, in very low yields ranging from 1 to 10%. To the best of our knowledge, only a report described the synthesis of 2,5-bis-(glutathion-S-yl)- $\alpha$ -methyldopamine from the oxidation of 5-(glutathion-S-yl)- $\alpha$ -methyldopamine, using sodium periodate in aqueous solution of 10% formic acid (yield not given) (12). Consequently, we decided to adapt our aforementioned electrochemical procedure to the synthesis of bi-adducts **4** and **5**, using catechol-thioether conjugates **1** and **2** as the starting materials, respectively. Our initial attempts to prepare the expected bi-adducts were disappointing, leading to low yields of the desired products ( $\leq 20\%$ ). As the electrolysis solution became dark purple, we attributed these results to the instability of the electrogenerated quinone-thioether species. As a consequence, subsequent addition of 2 equiv of thiol derivative did not result in the discoloration of the dark purple solution, in agreement with the formation of melanin-like polymers. So, to circumvent this problem, we decided to oxidize the catechol-thioether mono-adduct in the presence of thiol derivative. Then, we thought that the produced unstable quinone-thioether species would be trapped by thiol derivative before its decomposition.

However, to markedly simplify the synthesis of the bis-catechol-thioether conjugates **4** and **5**, we envisioned a two-step one-pot electrochemical procedure, which allowed the preparation of the bi-adduct, using HHMA as the starting material, without the need to isolate the mono-adduct intermediate (Scheme 2). For the synthesis of the bi-adduct **4** for example, the first experimental step consisted of the generation of the *o*-quinone species and consecutive addition of 2 equiv of glutathione, under the aforementioned experimental conditions. After the electrolysis solution became colorless, the catechol-thioether mono-adduct **1** was not isolated, but a third equiv of glutathione was added and, in a second step, the resulting solution was oxidized again at + 1.0 V versus Ag/AgCl. Then, the solution became

pale yellow and a coulometric value of 3.8 was found for n. To maintain a reducing medium during work-up, two additional equiv of glutathione were added at the end of the anodic oxidation. Finally, treatment of the electrolysis solution afforded, after semi-preparative reversed-phase HPLC (See Experimental Section), the catechol-thioether bi-adduct **4**, in 50% yield, together with 22% of the mono-adduct **1** (Scheme 2).

The same one-pot procedure allowed the isolation of bis-catechol-thioether conjugate **5**, in 44% yield, along with 22% of catechol-thioether mono-adduct **2**, isolated as a minor product (Scheme 2). In all cases, the presence of the catechol-thioether mono-adduct as a minor product could not be avoided, probably because redox interchange occurred between the redox systems present in the electrolysis solution.

**Synthesis of catechol-thioether conjugates of dopamine 6-8, 9 and 10.** When applied to dopamine, the aforementioned procedure used for the synthesis of catechol-thioether conjugates **1-3** led to the mono-adducts **6-8**, in yields ranging from 55 to 65%. Although the electrochemical preparation of conjugates **6-8** had been previously reported, the described procedure was poorly adapted for routine synthesis because the amount of isolated products was very low (36, 39-41). When applied to dopamine, the above procedure reported for the synthesis of bis-catechol-thioether conjugates **4** and **5** led to the bi-adducts **9** and **10** in 64 and 62% yield respectively, along with the corresponding mono-adducts **6** and **7**, isolated in 24 and 18% yields respectively. To date, these bi-adducts have been only described as by-products, in yields lower than 10%, in the course of the electrolysis of mono-conjugates (36, 39).

**Biological evaluation. Cytotoxicity profiles in OxyR<sup>-</sup> and OxyR<sup>+</sup> cells.** The cytotoxicity profiles of HHMA and its catechol-thioether conjugates **1-5** were characterized using simple *Escherichia coli* plate assays, and compared with those of dopamine and its related catechol-thioether conjugates **6-10**. The results are presented in Table 2.

The toxicity mediated by ROS (ROS-TOX) was detected in OxyR<sup>-</sup> cells deficient in the OxyR function, because they are unable to exert antioxidant defenses. In contrast, in OxyR<sup>+</sup> cells, ROS-TOX was inhibited due to the elimination of hydrogen peroxide by catalase and alkylhydroperoxide

reductase, both enzymes encoded by OxyR-regulated genes, so that the toxicity arising from binding of quinones with cellular nucleophiles (Q-TOX) could then be evidenced. Moreover, detection of Q-TOX was improved by adding to the assay tyrosinase, which promoted quinone formation.

As shown in Table 2, HHMA gave a positive response in the OxyR<sup>-</sup> assay (TC<sub>50</sub> = 0.75 μmol/plate), indicating high ROS-TOX induction, which was dose-dependent (Figure 3a). Furthermore, an increase in ROS-TOX (TC<sub>50</sub> = 0.5 μmol/plate) was observed in the presence of tyrosinase which catalyzed the oxidation to *o*-quinone (Figure 3a), suggesting that the oxidation of HHMA to the corresponding *o*-quinone opens the way for redox cycling, leading to the formation of ROS. Similarly, induction of ROS-TOX by HHMA was also enhanced in the presence of a nitric oxide-releasing agent promoting quinone formation (data not shown), a result in favor of an important role of the *o*-quinone species as redox active center generating ROS.

In contrast, the negative response in OxyR<sup>+</sup> assay, even in the presence of tyrosinase, was indicative of the absence of Q-TOX resulting from the high susceptibility of quinones toward endogenous nucleophiles. Note that the detection of ROS-TOX promoted by quinone redox cycling (OxyR<sup>-</sup>/tyrosinase assay) probably required the absence of Q-TOX (OxyR<sup>+</sup> assay/tyrosinase), which could mask the toxicity mediated by ROS. Accordingly, for catechol, tyrosinase induced potent Q-TOX to OxyR<sup>+</sup> cells (TC<sub>50</sub> = 0.1 μmol/plate), which prevented the detection of ROS-TOX in OxyR<sup>-</sup>/tyrosinase assay. Conversely, the tyrosinase-promoted induction of ROS-TOX by L-tyrosine, the amino-acid precursor of catecholamines, was easily detected because of the absence of Q-TOX induction (Table 2).

As HHMA did, catechol-thioether mono-conjugates **1** and **2** produced high ROS-TOX (TC<sub>50</sub> = 0.8 μmol/plate), which was enhanced in the presence of tyrosinase (Figure 3b), suggesting that the generated *o*-quinone-thioether can enter redox cycles leading to the formation of ROS, while no toxicity was evidenced with 5-cystein-S-yl mono-adduct **3** (Table 2). In this case, the redox activity of the generated quinone-thioether would be suppressed consecutively to a rapid intramolecular cyclization reaction: the cysteinyl amino group would condense with the quinone carbonyl to give a benzothiazolyl-like compound (dihydrobenzothiazine), as previously suggested by Monks *et al* (10-13, 16).

Although markedly less toxic than the corresponding mono-adduct **2** ( $TC_{50} = 0.8 \mu\text{mol/plate}$ ), 2,5-N-acetylcystein-S-yl bis-conjugate **5** was a good inducer of ROS-TOX ( $TC_{50} = 1.50 \mu\text{mol/plate}$ ). Once again, the significant increase in ROS-TOX promoted by tyrosinase ( $TC_{50} = 0.8 \mu\text{mol/plate}$ ) suggested ROS generation via the redox cycling of the generated quinone-thioether species (Figure 3b). To a lesser extent, the same conclusion could be drawn with the slightly toxic 2,5-glutathion-S-yl bis-conjugate **4**, for which the toxicity appeared only in the presence of tyrosinase ( $TC_{50} = 2.0 \mu\text{mol/plate}$ ).

Conversely to HHMA and its related catechol-thioether mono-adducts **1** and **2**, dopamine and its corresponding catechol-thioether mono-conjugates **6** and **7** were found to be poor inducers of ROS-TOX ( $TC_{50} = 4.0 \mu\text{mol/plate}$ ), while no Q-TOX was detected in the OxyR<sup>+</sup> assay (Table 2 and Figure 3). However, if these compounds were not very prone to autoxidation, catalytic oxidation by tyrosinase induced a significant increase of ROS-TOX ( $TC_{50}$  reached  $1.5 \mu\text{mol/plate}$  for mono-adduct **7**), indicating that the generated *o*-quinone or *o*-quinone-thioether could also enter redox cycles leading to the formation of ROS (Figure 3). As compound **3** did, 5-cystein-S-yl monoadduct **8** was toxic, neither to the OxyR<sup>-</sup> cells, nor to the OxyR<sup>+</sup> cells. However, in contrast to **3**, a weak induction of ROS-TOX was observed in the presence of tyrosinase ( $TC_{50} = 3.5 \mu\text{mol/plate}$ ). This could be explained by a difference in the rate of intramolecular cyclization reaction involving the cysteinyl amino group and the quinone carbonyl to give the dihydrobenzothiazine derivative. In other words, the quinone-thioether species generated from mono-adduct **6** would be more stable than that arising from the oxidation of mono-adduct **2**.

2,5-N-acetylcystein-S-yl bis-conjugate **10** was found to be a weak inducer of ROS-TOX, the toxicity being detected only in the presence of tyrosinase (Figure 3b), while no Q-TOX could be evidenced in the OxyR<sup>+</sup> assay (Table 2). Once again, a lower toxicity was found for the bi-adduct in comparison to that exhibited by the corresponding mono-adduct **6**. An unexpected behavior was observed with 2,5-glutathion-S-yl bis-conjugate **9** as it showed toxic effects at high doses (3 and 4  $\mu\text{mol/plate}$ ), irrespective of the presence or not of tyrosinase, whereas a low toxicity to OxyR<sup>+</sup> cells was observed.

Due to these results, further assays are needed to elucidate the toxicity profile of bis-conjugate **9** and, consequently, the data obtained from **9** are not reported in Table 2.

At this stage, we sought to justify the higher toxicity of HHMA and its related catechol-thioether conjugates **1-5** versus that of dopamine and its related catechol-thioether conjugates **6-10**. A comparison of the toxic effects with the corresponding oxidation potentials showed that there was no correlation between the detected toxicity and the oxidation potentials. For example, HHMA was found to be a much better inducer of ROS-TOX than dopamine, although they exhibited the same oxidation potential (+ 200 mV versus Ag/AgCl, in 0.2 mM phosphate buffered aqueous solution pH = 7.3). To date, we suspect that structural features mainly might account for the difference of toxicity between HHMA (or its related catechol-thioether conjugates) and dopamine (or its corresponding conjugates). In particular, HHMA possesses, on its side chain, a methyl group which creates an asymmetric center, together with a terminal secondary amine. No doubt these two methyl groups are sufficient to modify, not only the overall reactivity of this catecholamine, but also its affinity for cellular constituents through change in physicochemical parameters such as pKa and Log P. Accordingly, it would be interesting to synthesize HHMA and its related catechol-thioether conjugates in their enantiomerically and diastereomerically pure forms. These syntheses are currently in progress in our laboratory.

## Conclusion

The alarming increase in the recreational use of MDMA, and the multitude of adverse effects resulting from its misuse, require a complete understanding of the pharmacology and toxicology of this amphetamine derivative.

To date, there is a general consensus that MDMA metabolism, resulting in the formation of highly redox active metabolites, is required for the expression of MDMA-induced toxicity. However, the mechanism by which these metabolites initiate and propagate the toxic events remains to be thoroughly clarified.

This prompted us to develop a straightforward one-pot electrochemical procedure allowing the synthesis of catechol-thioether conjugates of MDMA **1-5**, in good yields and high degree of purity (99%), under environmentally friendly conditions. The utilization of electricity as energy instead of oxidative reagents, aqueous media instead of organic solvents, room temperature, one-pot operation and high atom economy, are of preeminent green advantages. To the best of our knowledge, at the exclusion of compound **2**, these catechol-thioether conjugates have never been prepared as yet. No doubt the synthesis of conjugates **1-5** will permit a comprehensive examination, both *in vitro* and *in vivo*, of their exact role in the diverse toxic effects of ecstasy.

To get an insight into the possible participation of the catechol-thioether conjugates **1-5** in the toxic events of MDMA, we have used simple bacterial assays, which could be predictive of the toxicological effects associated with these compounds against different targets *in vivo*. Furthermore, these assays could provide additional informations about the mechanism by which these metabolites could exert their toxic effects. In particular, the cytotoxicity profiles characterized by positive responses in OxyR<sup>-</sup> assay, which were reinforced in the presence of tyrosinase, supported the hypothesis of an oxidative mechanism dependent on the oxidation of HHMA and its catechol-thioether conjugates **1-5** to the corresponding *o*-quinone species. These could enter redox cycles, leading to the formation of ROS. The lack of positive responses in OxyR<sup>+</sup> assay corroborated this hypothesis as these quinonoid species failed to induce Q-TOX arising from binding with cellular nucleophiles. Dopamine and its corresponding

quinol-thioether conjugates **6-10** showed a low susceptibility to spontaneous oxidation. However, tyrosinase, which promoted quinone formation, was able to enhance ROS-TOX, in agreement with *o*-quinone species acting as redox active centers generating ROS.

From a general toxicological point of view, the catecholamine derivatives constitute a specific class of quinol derivatives because simple catechol derivatives are rather prone to Q-TOX, due to the high susceptibility of the generated *o*-quinone to give Michael addition reaction with cellular nucleophiles. Recently, we have also described another behavior of quinol derivatives possessing a pyrogallol arrangement, for which high ROS-TOX was detected in OxyR<sup>-</sup> cells. However, in the presence of agents promoting quinone formation such as nitric oxide, a significant decrease of ROS-TOX was observed, suggesting that the nitric oxide promoted two-electron oxidation process would compete with the one-electron oxidation reactions initiating ROS-TOX phenomena. In this case, the results in the OxyR<sup>-</sup> assay showed a good correlation with those given by the MTT assay performed *in vitro* on murine HT-22 hippocampal cell cultures, thus allowing to approach the toxicity mechanism of neuroprotective agents (29). Obviously, the amino side chain of catecholamine derivatives plays a major role in the development of ROS-TOX involving quinonoid species. This point would merit thorough further investigations.

Finally, the last question that remains is the relevance of our *in vitro* results to the *in vivo* situation. Despite the fact that HHMA and some catechol-thioether conjugates are present *in vivo* (17, 23), they have not been undoubtedly established as *in vivo* toxic metabolites of MDMA. Furthermore, several other pathways could also contribute to the overall MDMA toxicity, such as mechanism-based inhibition of CYP2D6, the main P450 enzyme responsible for the demethylenation of MDMA. In addition, the fraction of HHMA that undergoes oxidation to the *o*-quinone species, relative to the fraction that undergoes catechol *o*-methyltransferase (COMT) mediated *o*-methylation is unknown (17). However, catechol estrogens undergo significant oxidation coupled to GSH conjugation (28-50%), even under conditions optimized for COMT-catalyzed *o*-methylation (presence of ascorbate) (55). Taking all together, it can be suggested that HHMA, and catechol-thioether conjugates **1-5**, could participate in the toxic effects of MDMA through the efficiency of redox active quinonoid centers generating ROS.

**Acknowledgment.** We thank Dr. M.-B. Fleury, Emeritus Professor at the Paris Descartes University, for fruitful discussions. We thank also Carmen Navarro for her technical assistance. This Research was made possible thanks to the joint financial support of the Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie (MILDT) and the Institut National de la Santé et de la Recherche Médicale (INSERM). (Appel à projets commun 2004 MILDT-INSERM « Recherche sur les drogues et la toxicomanie »). A.F. thanks MILDT together with INSERM for a PhD grant.

**Supporting Information Available.** Analytical HPLC chromatograms together with  $^1\text{H}$  NMR spectra for all catechol-thioether conjugates **1-10**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Green, A.R., Mehan, A.O., Elliot, J.M., O'Shea, E., and Colado, M.I. (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol. Rev.* 55, 463-508 and references therein.
- (2) Cole, J.C., and Sumnall H.R. ( 2003) Altered states: the clinical effects of ecstasy. *Pharmacol. Ther.* 98, 35-58.
- (3) Lyles, J., and Cadet, J.L. (2003) Methylenedioxymethamphetamine (MDMA, Ecstasy) neurotoxicity: cellular and molecular mechanisms. *Brain Res. Rev.* 42, 155-168 and references therein.
- (4) Morton, J. (2005) Ecstasy: pharmacology and neurotoxicity. *Curr. Opinion Pharmacol.* 5, 79-86.
- (5) Ricaurte, G.A., and McCann, U.D. (2005) Recognition and management of complications of new recreational drug use. *Lancet*, 365, 2137-2145.
- (6) Baumann, M.H., Wang, X., and Rothman, R.B. (2007) 3,4-methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. *Psychopharmacology*, 189, 407-424.
- (7) Milroy, C.M., Clark, J.C., and Forrest, A.R.W. (1996) Pathology of deaths associated with "ecstasy" and "eve" misuse. *J. Clin. Pathol.* 49, 149-153.
- (8) Walubo, A., and Seger, D. (1999) Fatal multi-organ failure after suicidal overdose with MDMA, "ecstasy": case report and review of the literature. *Hum. Exp. Toxicol.* 18, 119-125.
- (9) Fineschi, V., Centini, F., Mazzeo, E., Turillazzi, E. Adam (MDMA) and Eve (MDA) misuse : an immunohistochemical study on three fatal cases. (1999) *Forensic Sci. Int.* 104, 65-74.

- (10) Miller, R.T., Lau, S., and Monks, T.J. (1995) Metabolism of 5-(glutathion-S-yl)- $\alpha$ -methyldopamine following intracerebroventricular administration to male Sprague-Dawley rats. *Chem. Res. Toxicol.* 8, 634-641.
- (11) Miller, R.T., Lau, S., and Monks, T.J. (1996) Effects of intracerebroventricular administration of 5-(glutathion-S-yl)- $\alpha$ -methyldopamine on brain dopamine, serotonin and norepinephrine concentrations in male Sprague-Dawley rats. *Chem. Res. Toxicol.* 9, 457-465.
- (12) Miller, R.T.; Lau, S.; Monks, T.J. (1997) 2,5-bis-(glutathion-S-yl)- $\alpha$ -methyldopamine, a putative metabolite of ( $\pm$ )-3,4-methylenedioxy amphetamine, decreases brain serotonin concentrations. *Eur. J. Pharmacol.* 323, 173-180.
- (13) Bai, F.B., Lau, S.S., and Monks, T.J. (1999) Glutathione and N-acetylcysteine conjugates of  $\alpha$ -methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxymethamphetamine-mediated neurotoxicity. *Chem. Res. Toxicol.* 12, 1150-1157.
- (14) Bai, F., Jones, D.C., Lau, S.S., and Monks, T.J. (2001) Serotonergic neurotoxicity of 3,4-( $\pm$ )-methylenedioxyamphetamine and 3,4-( $\pm$ )-methylenedioxymethamphetamine (ecstasy) is potentiated by inhibition of  $\gamma$ -glutamyl transpeptidase. *Chem. Res. Toxicol.* 14, 863-870.
- (15) Jones, D.C., Lau, S.S., and Monks, T.J. (2004) Thioether metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine inhibit human serotonin transporter (hSERT) function and simultaneously stimulate dopamine uptake into hSERT-expressing SK-N-MC cells. *J. Pharmacol. Exp. Ther.* 311, 298-306.
- (16) Monks, T.J., Jones, D.C., Bai, F., and Lau, S.S. (2004) The role of metabolism in 3,4-( $\pm$ )-methylenedioxyamphetamine and 3,4-( $\pm$ )-methylenedioxymethamphetamine (Ecstasy) toxicity. *Ther. Drug Monit.* 26, 132-136.

- (17) Jones, D.C., Duvauchelle, C., Ikegami, A., Olsen, C.M., Lau, S.S., de la Torre, R. and Monks, T.J. (2006) Serotonergic Neurotoxic Metabolites of Ecstasy Identified in Rat Brain. *J. Pharmacol. Exp. Ther.* 313, 422-431.
- (18) Capela, J.P., Meisel, A., Abreu, A.R., Branco, P.S., Ferreira, L.M., Lobo, A.M., Remiao, F., Bastos, M.L., and Carvalho, F. (2006) Neurotoxicity of ecstasy metabolites in rat cortical neurons, and influence of hyperthermia. *J. Pharmacol. Exp. Ther.* 316, 53-61.
- (19) Carvalho, M., Remiao, F., Milhazes, N., Borges, F., Fernandes, E., Carvalho, F., and Bastos, M.L. (2004) The toxicity of N-methyl- $\alpha$ -methyldopamine to freshly isolated rat hepatocytes is prevented by ascorbic acid and N-acetylcysteine. *Toxicology*, 200, 193-203.
- (20) Carvalho, M., Milhazes, N., Remiao, F., Borges, F., Fernandes, E., Amado, F., Monks, T.J., Carvalho, F., and Bastos, M.L. (2004) Hepatotoxicity of 3,4-methylenedioxyamphetamine and  $\alpha$ -methyldopamine in isolated rat hepatocytes: formation of glutathione conjugates. *Arch. Toxicol.* 78, 16-24.
- (21) Carvalho, M., Hawthorth, G., Milhazes, N., Borges, F., Monks, T.J., Fernandes, E., Carvalho, F., and Bastos, M.L. (2002) Role of metabolites in MDMA (ecstasy)-induced nephrotoxicity: an in vitro study using rat and human renal proximal tubular cells. *Arch. Toxicol.* 76, 581-588.
- (22) Carvalho, M., Remiao, F., Milhazes, N., Borges, F., Fernandes, E., do Céu Monteiro, M., Goncalves, M.J., Seabra, V., Amado, F., Carvalho, F., and Bastos, M.L. (2004) Metabolism is required for the expression of Ecstasy-induced cardiotoxicity in vitro. *Chem. Res. Toxicol.* 17, 623-632.
- (23) Segura, M., Jordi, O., Farré, M., McLure, J.A., Pujadas, M., Pizarro, N., Llebaria, A., Joglar, J., Roset, P.N., Segura, J., and de la Torre, R. (2001) 3,4-Dihydroxymethamphetamine (HHMA). A major in vivo 3,4-methylenedioxyamphetamine (MDMA) Metabolite in Humans. *Chem. Res. Toxicol.* 14, 1203-1208.

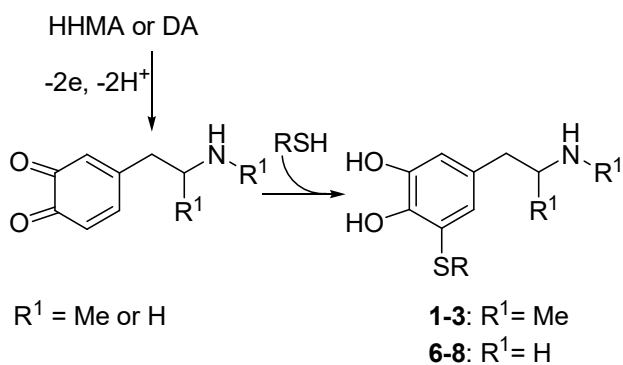
- (24) Esteban B., O'Shea, E., Camarero, J., Sanchez, V., Green, A.R., Colado, M.I. (2001) 3, 4-methylenedioxymethamphetamine induces monoamine release, but not toxicity, when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. *Psychopharmacology*, 154, 251-260.
- (25) De La Torre, R., Farré, M., Monks, T.J., Jones, D. (2005) Response to Sprague and Nichols : Contribution of metabolic activation to MDMA neurotoxicity. *Trends Pharmacol. Sci.* 26, 60-61.
- (26) Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J. (2000), Role of quinones in toxicology. *Chem. Res. Toxicol.* 13, 135-160.
- (27) Martínez, A., Urios, A., Blanco, M. (2000) Mutagenicity of 80 chemicals in *Escherichia coli* tester strains IC203, deficient in OxyR, and its *oxyR*<sup>+</sup> parent WP2 *uvrA*/pKM101: detection of 31 oxidative mutagens. *Mutat. Res.* 467, 41-53.
- (28) Urios, A., López-Gresa, M.P., González, M.C., Primo, J., Martínez, A., Herrera, G., Escudero, J.C., O'Connor, J.E., Blanco, M. (2003) Nitric oxide promotes strong cytotoxicity of phenolic compounds against *Escherichia coli*: the influence of antioxidant defenses. *Free Radic. Biol. Med.* 35, 1373-1381.
- (29) Urios, A., Largeron, M., Fleury, M.-B., Blanco, M. (2006) A convenient approach for evaluating the toxicity profile of in vitro neuroprotective alkylaminophenol derivatives. *Free Radic. Biol. Med.* 40, 791-800.
- (30) Christman, M.F., Morgan, R.W., Jacobson, F.S., Ames, B.N. (1985) Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. *Cell* 41, 753-762.

- (31) Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossa, R.A., Storz, G. (2001) DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* *183*, 4562-4570.
- (32) Borgman, R.J. Baylor, M.R., McPhillips, J.J., Stitzel, R.E. (1974)  $\alpha$ -Methyldopamine derivatives. Synthesis and pharmacology. *J. Med. Chem.* *17*, 427-430.
- (33) Morgan, P.H., Beckett, A.H. (1975) Synthesis of some N-oxygenated products of 3,4-dimethoxyamphetamine and its N-alkyl derivatives. *Tetrahedron*, *31*, 2595-2601.
- (34) Cannon, J.G., Perez, Z., Long, J.P., Rusterholtz, D.B., Flynn, J.R., Costall, B., Fortune, D.H., Naylor, R.J. (1979) N-alkyl derivatives of ( $\pm$ )- $\alpha$ -methyldopamine. *J. Med. Chem.* *22*, 901-907.
- (35) Milhazes, N., Cunha-Oliveira, T., Martins, P., Garrido, J., Oliveira, C., Rego, Cristina A. and Borges, F. (2006) Synthesis and cytotoxic profile of 3,4-methylenedioxymethamphetamine ("Ecstasy") and its metabolites on undifferentiated PC12 cells: a putative structure-toxicity relationship. *Chem. Res. Toxicol.* *19*, 1294-1304.
- (36) Zhang, F., Dryhurst, G. (1995) Influence of glutathione on the oxidation chemistry of the catecholaminergic neurotransmitter dopamine. *J. Electroanal. Chem.* *398*, 117-128.
- (37) Zheng, Z.-B.; Nagai, S.; Iwanami, N.; Kobayashi, A.; Natori, S.; Sankawa, U. (1999) Inhibition effects of 5-S-Glutathionyl-N- $\beta$ -alanyl-L-dopa analogues against Src protein Tyrosine Kinase. *Chem. Pharm. Bull.* *47*, 777-782.
- (38) Picklo, M.J., Amarnath, V., Graham, D.G. and Montine, T.J. (1999) Endogenous catechol thioethers may be pro-oxidant or antioxidant. *Free Radic. Biol. Med.* *27*, 271-277.
- (39) Shen, X.-M., Xia, B., Wrona, M.Z. and Dryhurst, G. (1996) Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteiny conjugates of dopamine: possible metabolites of relevance to Parkinson's disease. *Chem. Res. Toxicol.* *9*, 1117-1126.

- (40) Xu, R.; Huang, X.; Kramer, K.J. ; Hawley, M.D. (1996) Characterization of products from the reactions of dopamine quinone with N-acetylcysteine. *Bioorg. Chem.* 24, 110-126.
- (41) Zhang, F.; Dryhurst, G.; (1994) Effects of L-cysteine on the oxidation chemistry of dopamine: new reaction pathways of potential relevance to idiopathic Parkinson's disease. *J. Med. Chem.* 37, 1084-1098.
- (42) Blanco, M., Urios, A., and Martínez, A. (1998) New *Escherichia coli* WP2 tester strains highly sensitive to reversion by oxidative mutagens. *Mutat. Res.* 413, 95-101.
- (43) Land, E.J., Ramsden, C.A., Riley, P.A. (2003) Tyrosinase autoactivation and the chemistry of *ortho*-quinone amines. *Acc. Chem. Res.* 36, 300-308 and references therein.
- (44) Ito, S.; Prota, G. (1977) A facile one-pot synthesis of cysteinyl-dopas using mushroom tyrosinase. *Experientia* 33, 1118-1119.
- (45) Ito, S.; Fugita, K.; Yoshioka, M.; Sienko, D.; Nagatsu, T. (1986) Identification of 5-S and 2-S-cysteinyl-dopamine and 5-S-glutathionyl-dopamine formed from dopamine by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 375, 134-140.
- (46) Ito, S.; Prota, G. (1977) Novel reaction of cysteine with phenolic amino-acids in hydrobromic acid: reversible formation of 3-cystein-S-yltyrosine and cystein-S-yl-dopas. *J. Chem. Soc. Chem. Commun.* 251-252.
- (47) Ito, S.; Inoue, S.; Yamamoto, Y.; Fujita, K. (1981) Synthesis and antitumor activity of cysteinyl-3,4-dihydroxyphenylalanines and related compounds. *J. Med. Chem.* 24, 673-677.
- (48) Chioccare, F.; Novellino, E. (1986) A convenient one step synthesis of 5-cysteinyl-S-yl-dopa using ceric ammonium nitrate. *Synth. Commun.* 16, 967-971.

- (49) LARGERON, M., LOCKHART, B., PFEIFFER, B., FLEURY, M.-B. (1999) Synthesis and in vitro evaluation of new 8-amino-1,4-benzoxazine derivatives as neuroprotective antioxidants. *J. Med. Chem.* 42, 5043-5052.
- (50) LARGERON, M., FLEURY, M.-B. (2000) Oxidative deamination of benzylamine by electrogenerated quinonoid systems as mimics of amine oxidoreductases. *J. Org. Chem.* 65, 8874-8881.
- (51) XU, D., CHIARONI, A., LARGERON, M. (2005) A one pot regiospecific synthesis of highly functionalized 1,4-benzodioxin derivatives from an electrochemically induced Diels-Alder reaction. *Org. Lett.* 7, 5273-5276.
- (52) SHEN, X.-M.; DRYHURST, G. (1996) Oxidation chemistry of (-)-norepinephrine in the presence of L-cysteine. *J. Med. Chem.* 39, 2018-2029.
- (53) HUANG, X.; XU, R.; HAWLEY, M.D.; HOPKINS, T.L.; KRAMER, K.J. (1998) Electrochemical oxidation of N-acyldopamines and regioselective reactions of their quinones with N-acetylcysteine and thiourea. *Arch. Biochem. Biophys.* 352, 19-30.
- (54) The synthesis of compound **1** has been the subject of a preliminary report. See: LARGERON, M.; NEUDÖRFFER, A.; GRAMOND, J.-P.; FLEURY, M.-B. (2003) Biomimetic electrochemical synthesis of quinol-thioether conjugates: their implication in the serotonergic neurotoxicity of amphetamine derivatives. *Ann. Pharm. Fr.* 61, 164-172 (in French).
- (55) BUTTERWORTH, M., LAU, S.S., MONKS, T.J. (1996) 17 $\beta$ -Estradiol metabolism by hamster hepatic microsomes: comparison of catechol estrogen *o*-methylation with catechol estrogen oxidation and glutathione conjugation. *Chem. Res. Toxicol.* 9, 793-799.

**Table 1.** One-pot synthesis of catechol-thioether mono-adducts **1-3** and **6-8**<sup>a</sup>



R <sup>1</sup>	RSH	Product	yield% <sup>b</sup>
Me	Glutathione	<b>1</b>	64
Me	NAC	<b>2</b>	66
Me	Cysteine	<b>3</b>	50
H	Glutathione	<b>6</b>	65
H	NAC	<b>7</b>	55
H	Cysteine	<b>8</b>	63

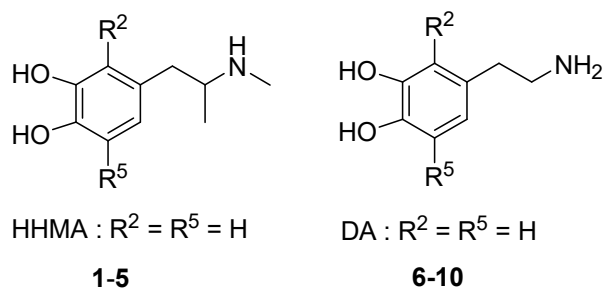
<sup>a</sup>Reagents and conditions: HHMA or DA = 1mM, RSH = 2 mM, 0.2 M HCl, rt, Pt anode (E = + 1.0 V vs Ag/AgCl), 30 min. <sup>b</sup>Yields refer to isolated products (after semi-preparative reversed-phase HPLC, degree of purity: 99%). NAC: N-acetylcysteine. DA:dopamine

## Figure Legends

**Figure 1.** Chemical Structures of Synthesized Catechol-Thioether Conjugates **1-10**

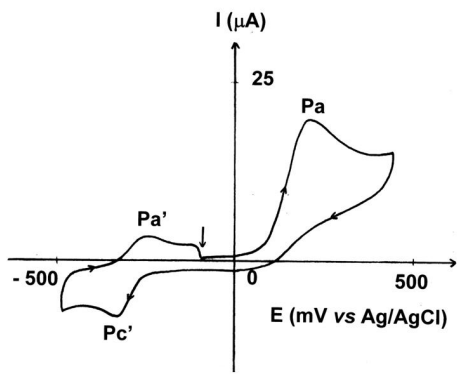
**Figure 2.** Cyclic Voltammogram of HHMA (1 mM) at a platinum electrode a) in deaerated phosphate buffered aqueous solution (pH 7.3),  $v = 200 \text{ mVs}^{-1}$ ; b) in deaerated 0.2 M HCl aqueous solution,  $v = 10 \text{ mVs}^{-1}$ . Arrowheads indicate the direction of the potential sweep. The vertical arrow indicates the initial potential point.

**Figure 3.** Dose-response curves: survival of OxyR<sup>-</sup> cells after exposure a) to HHMA and dopamine; where indicated, tyrosinase (TYR) was added to the assay (50 units/plate); b) to catechol-thioether conjugates **2**, **5**, **7** and **10**; tyrosinase (TYR) was added to the assay (50 units/plate). Data are the mean  $\pm$  SD of at least three experiments.

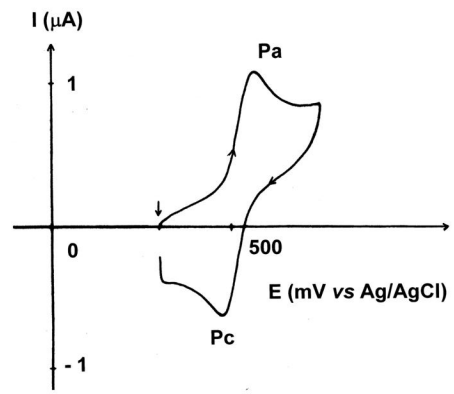


- 1, 6 :  $R^2 = H$ ;  $R^5 = \text{glutathion-S-yl}$
- 2, 7 :  $R^2 = H$ ;  $R^5 = \text{N-acetylcystein-S-yl}$
- 3, 8 :  $R^2 = H$ ;  $R^5 = \text{cystein-S-yl}$
- 4, 9 :  $R^2 = R^5 = \text{glutathion-S-yl}$
- 5, 10 :  $R^2 = R^5 = \text{N-acetylcystein-S-yl}$

Figure 1

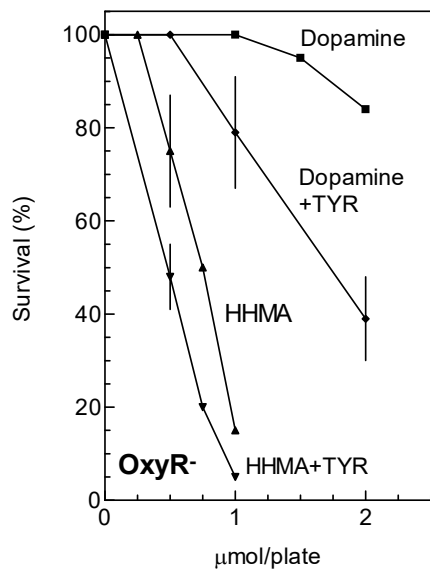


2a

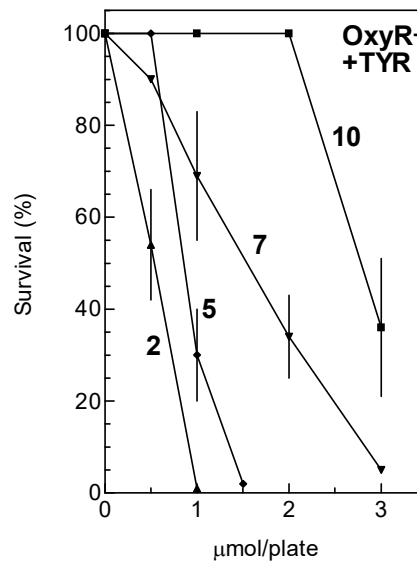


2b

Figure 2

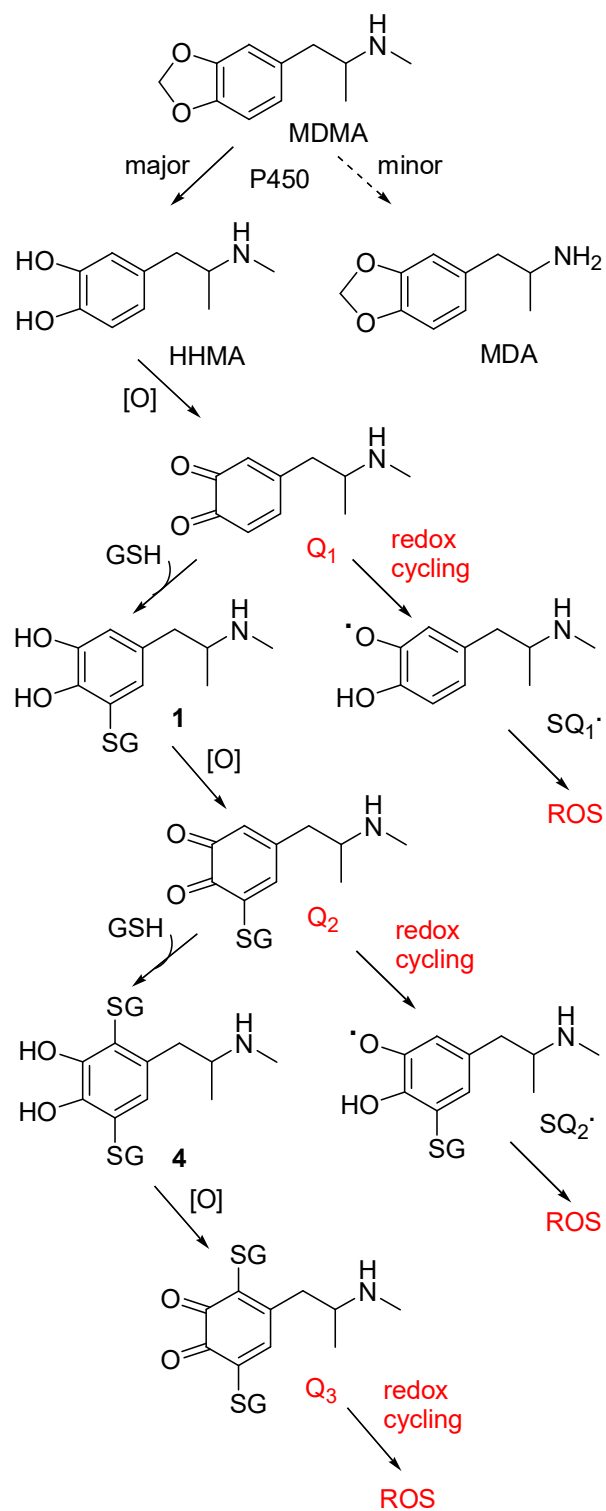


3 a

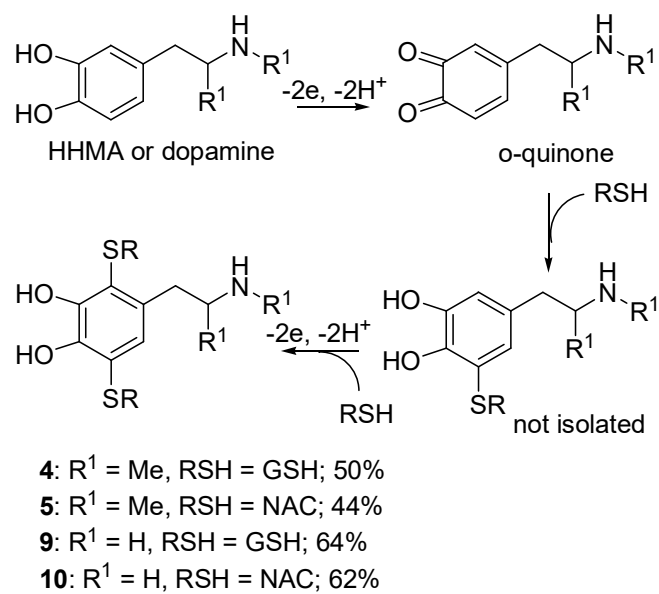


3 b

Figure 3



**Scheme 1.** Proposed Routes for MDMA Metabolism to Redox Active Catechol-Thioether Conjugates in Humans



**Scheme 2.** Two-Step One-Pot Electrochemical Synthesis of bis-Catechol-Thioether Conjugates **4**, **5**, **9** and **10**.