Quercetin as the Active Principle of *Hypericum hircinum* Exerts a Selective Inhibitory Activity against MAO-A: Extraction, Biological Analysis, and Computational Study

Franco Chimenti,[†] Filippo Cottiglia,[‡] Leonardo Bonsignore,[‡] Laura Casu,[‡] Mariano Casu,[§] Costantino Floris,[§] Daniela Secci,*[,†] Adriana Bolasco,[†] Paola Chimenti,[†] Arianna Granese,[†] Olivia Befani,[⊥] Paola Turini,[⊥] Stefano Alcaro,[∥] Francesco Ortuso,[∥] Giuseppe Trombetta,[▽] Alberto Loizzo,[▽] and Irene Guarino[▽]

Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy, Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy, Dipartimento di Scienze Chimiche, Università di Cagliari, Complesso di Monserrato, SS 554, Km 4.5, 09042 Monserrato (CA), Italy, Dipartimento di Scienze Biochimiche "A. Rossi Fanelli" and Centro di Biologia Molecolare del CNR Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy, Dipartimento di Scienze Farmaco Biologiche "Complesso Ninì Barbieri", Università degli Studi di Catanzaro "Magna Graecia", 88021 Roccelletta di Borgia (CZ), Italy, and Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Received January 12, 2006

The methanol extract from *Hypericum hircinum* leaves exhibited in vitro inhibition of monoamine oxidases (MAO). Bioassay-guided fractionation led to the isolation of quercetin and five compounds identified for the first time from *H. hircinum*. Quercetin was the only compound with a selective inhibitory activity against MAO-A, with an IC_{50} value of 0.010 μM. To explain MAO selective inhibition at the molecular level, a computational study was carried out by conformational search and docking techniques using recently determined crystallographic models of both enzymatic isoforms. An in vivo study in mice was carried out using the forced swimming test in order to elucidate the behavioral effects of quercetin.

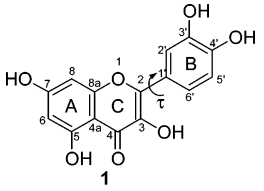
Quercetin **1** (3,5,7,3′,4′-pentahydroxyflavone, Figure 1) is widely distributed in nature, and its structure has been elucidated by theoretical, experimental, and semiempirical studies, the latter addressed to the structural, conformational, and electronic features of this compound.[1] More recently a high-level ab initio and molecular dynamics simulation on flavonoids including quercetin was performed.[2] Cocrystals of quercetin and different enzymes, such as the hematopoietic cell kinase,[3] phosphoinositide 3-kinase,[4] and quercetin 2,3-dioxygenase,[5] have been determined and deposited in the Protein Data Bank (PDB). In these studies, planar conformations with torsion angles τ (6′−1′−2−3) of 180° or 0° were observed, especially in the crystal models. Additional nonplanar conformations close to the flat structures with τ of about 180° or 0° ± 30° have been reported by theoretical methods with details about the energy barriers.[2]

Various studies have shown that quercetin has wide-ranging pharmacological actions[7−13] and acts as a COMPT/MAO inhibitor.[8]

The genus *Hypericum* (Guttiferae) comprises a number of species with many different biological activities, such as antimicrobial[14] and antidepressant activities.[15] *Hypericum perforatum* extracts are widely used for the treatment of mild to moderate depression, and hypericin is considered the most active constituent.[16] Nevertheless, the opinion of many researchers is that the antidepressant effects are due to more than one constituent,[6,17,18] such as xanthones,[19] flavonoids,[6] and hyperforin.[18]

Some authors have recently reported the monoamine oxidase inhibitory activity of some *Hypericum* species[20] and of benzopyrans isolated from their extracts.

A study on other *Hypericum* species could provide important information about the observed MAO inhibitory activity and may provide a source of new compounds involved in MAO inhibition.



1

Figure 1. Structure of quercetin with numbering scheme, ring labels, and the most important torsional definition.

H. hircinum L. is a shrub that grows from Liguria to Sicily, including Sardinia and Corsica. The plant is widely used in Lucanian folk medicine for the treatment of cough.[21] There is no report on the MAO inhibitory activity of *H. hircinum*. It has been reported that the leaves contain chlorogenic acid, quercetin, quercetrin, mangiferin, and biapigenin, but no phloroglucinol compounds such as hyperforins or hypericins.[14] As part of our continuing research on MAO inhibitors,[22a,b] we now describe the MAO inhibitory activity of the MeOH extract of *H. hircinum* leaves along with the identification of six compounds that, except for quercetin, are reported for the first time as components of the aerial parts.

Quercetin showed a selective MAO-A inhibitory activity in the nanomolar range, and for this reason, following our previous experience in this field,[22b] we carried out a computational study to justify the selective inhibition. Conformational search and docking experiments were performed with crystallographic MAO models. Statistical thermodynamics and distance analysis allowed the estimation of state functions of the complexation process and identification of the important residues involved in the selective recognition of quercetin in the MAO enzymatic clefts.

In addition, an in vivo study in mice was carried out in order to elucidate the behavioral effects of quercetin using the forced swimming test (FST), in which immobility posture observed is assumed to be a depressive-like parameter.[23]

Results and Discussion

The ability of the total MeOH crude extract of *H. hircinum* leaves to inhibit MAO in the presence of kynuramine as a substrate was

* To whom correspondence should be addressed. E-mail: daniela.secci@uniroma1.it. Tel and Fax: +39 06 4991 3763.
[†] Dip. S.C.T.S.B.A., University of Rome "La Sapienza".
[‡] Dip. Farmaco Chimico Tecnologico, University of Cagliari.
[§] Dip. Scienze Chimiche, University of Cagliari.
[⊥] Dip. Scienze Biochimiche "A.Rossi Fanelli", University of Rome "La Sapienza".
[∥] Dip. Scienze Farmaco Biologiche, University of Catanzaro.
[▽] Istituto Superiore di Sanità.

10.1021/np060015w CCC: $33.50
Published on Web 05/24/2006

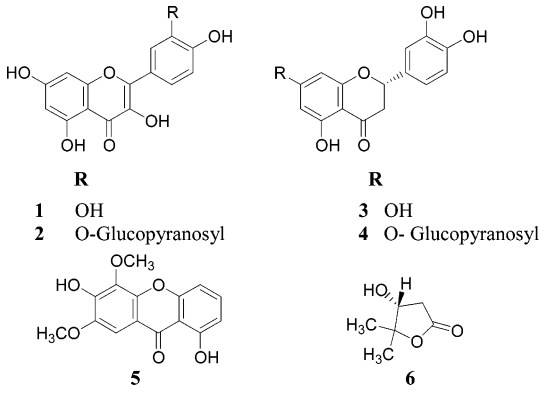


Figure 2. Structures of the isolated compounds from *H. hircinum*.

first evaluated by a preliminary assay performed with 10, 30, and 50 μL of the extract. The inhibitory activity showed values in the range 27−49%. After this assessment the extract was first partitioned between CH_2Cl_2 and H_2O, and the aqueous layers were fractionated by VLC, subsequent CC, and HPLC (RP-18) to afford quercetin (**1**), eriodictyol (**3**), 1,6-dihydroxy-5,7-dimethoxyxanthone (**5**), and (4*R*)-4-hydroxy-5,5-dimethyldihydrofuran-2-one (**6**) from the CH_2Cl_2 phase and quercetin-3′-*O*-*β*-D-glucopyranoside (**2**) and eriodictyol-7-*O*-*β*-D-glucopyranoside (**4**) from the aqueous soluble part (Figure 2). The physical and spectroscopic data agree with those reported,[24−28] and although compound **6** has been obtained by synthesis,[24] it is isolated for the first time as a natural product. Complete NMR data for compound **5** are reported.[29]

The compounds were tested to determine the activity toward MAO-A and MAO-B in the presence of the specific substrates serotonin and benzylamine, respectively. The only compound with MAO activity is quercetin, identified in the CH_2Cl_2 part of the extract, with an IC_{50} MAO-B of 20 μM and IC_{50} MAO-A of 0.010 μM, indicating a high A selectivity (selectivity index, $SI_{MAO-B/MAO-A} = 2000$) for the inhibitory activity of this compound with respect to the reference compound toloxatone ($K_i = 1.8$ μM).[30]

It is interesting to point out that quercetin acts through the reversible mode, as shown by a 24-h dialysis in a cold room against a 0.1 M potassium phosphate buffer (pH 7.2) capable of restoring 90−100% of the activity of the enzyme involved in the catalytic site.

A computational study was carried out with the aim of understanding the differences in the interaction of quercetin with the hMAO isoforms. The study started with a model of quercetin performed by molecular mechanics methods and followed by docking simulations using the most recent hMAO crystallographic structures available in the PDB,[31,32] with a previously reported method.[33] Using methods of statistical thermodynamics and molecular graphics, it was possible to estimate the state functions of the recognition process and identify the most important interactions between the residues and the cofactor within the enzymatic cleft. The free energy of complexation, computed by means of the MOLINE method,[34] was in agreement with data on experimental inhibition and confirmed the preferred MAO-A recognition in both configurational ensembles obtained after an MC docking search and full energy minimization (see Table S1 of the Supporting Information). The known major structural differences in the clefts of the two enzymes[35] and their different volume sizes[32] were considered the most plausible reason for the preferential recognition of quercetin in hMAO-A. Quercetin fits better in the hMAO-A than in the hMAO-B cleft, with more intermolecular hydrogen bonds and $\pi-\pi$ interaction.

The regulation of MAO activity appears to play a central role in several psychiatric and neurological disorders and plays an important role in the metabolism of monoamine neurotransmitters. MAO-A inhibitor drugs have been accepted in the treatment of depressive states.[36] It has been suggested that the antidepressive-like activity of natural flavonoids such as quercetin was due, at least partially, to their MAO-inhibitory properties,[37,38] though only a few studies compare their neurochemical effects obtained in vitro with the behavioral profile displayed in animal models.

In Porsolt's FST, a model extensively used to study depressive behavior in rodents and to assess the pharmacological profile of antidepressive drugs, MAO inhibitors may improve the immobility elicited by the test, though the antidepressant-like behavioral responses of quercetin are rather controversial.[39]

Our experiments showed no significant differences in the duration of immobility when observed between the naïve control group and the CMC (carboxy methyl cellulose) vehicle group. Acute ip administration of quercetin at doses of 1 and 10 mg/kg failed to induce behavioral effects and did not affect the duration of immobility compared to both naïve controls and CMC vehicle mice. However, the 100 mg/kg dose of quercetin significantly increased this duration with respect to all experimental groups (Figure 5).

This effect may be explained in part by a possible sedative effect shown by quercetin at a higher dose in the FST, as already

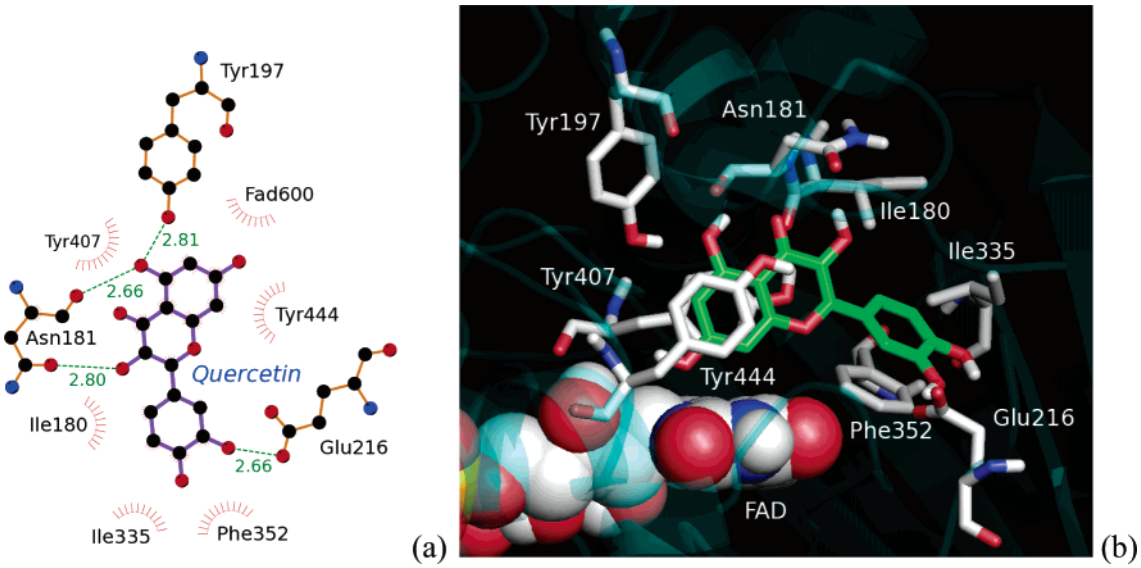


Figure 3. Lowest interaction energy configuration of quercetin MAO-A complex in Ligplot (a) and Pymol (b) representations. The ligand is depicted in polytube green carbons, residues identified by Ligplot are labeled, and the rest of the protein is reported in transparent light cyan ribbon rendering and the FAD in CPK model.

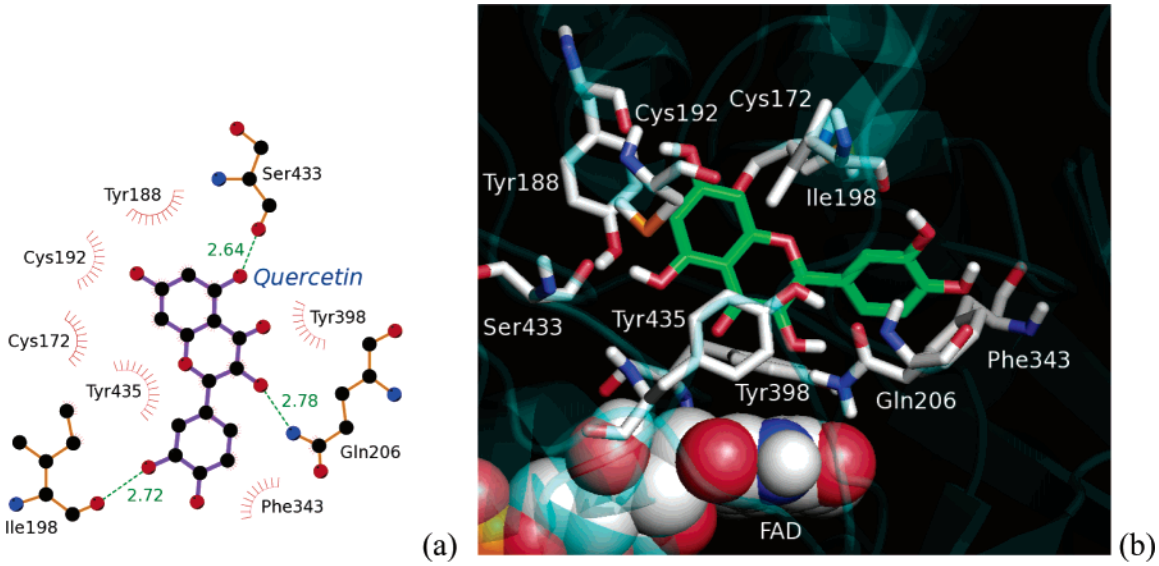


Figure 4. Lowest interaction energy configuration of quercetin MAO-B complex in Ligplot (a) and Pymol (b) representations. The ligand is depicted in polytube green carbons, residues identified by Ligplot are labeled, and the rest of the protein is reported in transparent light cyan ribbon rendering and the FAD in CPK model.

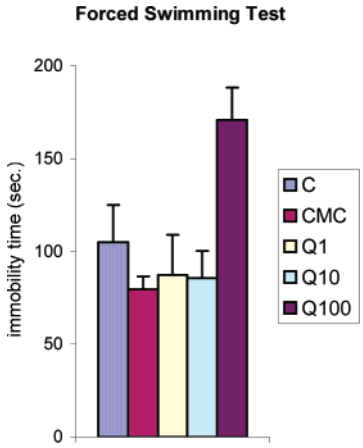


Figure 5. Effect of quercetin (1, 10, and 100 mg/kg) in the forced swimming test. Data are expressed as mean ± SEM values. *P < 0.05 as compared with control mice and 1−10 mg/kg doses.

reported.[40] It must also be considered that quercetin and other flavonoids have recently been reported to bind selectively with high affinity to the central benzodiazepine receptor and may exert a modulatory activity on the ionotropic GABA receptor,[41,42] suggesting a possible dual effect in behavioral animal models supported by a different neurochemical pathway.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a GBC Cintra 5 spectrophotometer. Melting points were determined on a Köfler apparatus and are uncorrected. EIMS spectra were taken on a QMD 1000 instrument at 70 eV using a direct inlet system. NMR spectra were recorded at 25 °C on a Varian UNITY INOVA spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Compounds were measured in C_5D_5N and CD_3OD, and the spectra referenced against residual nondeuterated solvents. Column chromatography was carried out under TLC monitoring using silica gel (40−63 μm, Merck) and Sephadex LH-20 (25−100 μm, Pharmacia). For vacuum liquid chromatography (VLC) silica gel (40−63 μm, Merck) was used. TLC was performed on silica gel 60 F_{254} or RP-18 F_{254} (Merck). HPLC was done by means of a Hewlett-Packard 1050 instrument. The column was a 250 × 10 mm Spherisorb S5 ODS 2, particle size 5 μm (Waters). UV detection wavelength was 360 nm.

Plant Material, Extraction, and Fractionation of the Extract. The leaves of *H. hircinum* were collected around Burcei (Cagliari, Sardinia, Italy) in July 2003. The plant material was identified by Prof. Bruno De Martis (University of Cagliari, Dipartimento di Scienze Botaniche), and a voucher specimen (No. 0201) is deposited in the Herbarium of the Dipartimento Farmaco Chimico Tecnologico, University of Cagliari, Italy.

Air-dried and powdered leaves of *H. hircinum* (210 g) were ground and extracted with petroleum ether (5 L) by percolation at room temperature to give 9 g of dried extract. The remaining plant material was then extracted with MeOH (4 L), giving 62 g of crude extract.

The MeOH extract was partitioned between CH_2Cl_2 and H_2O. The organic phase (12 g) was subjected to VLC (silica gel, 90 g, 40−63 μm) using a step gradient of hexane−CH_2Cl_2/EtOAc (9:1:0 to 0:1:9, 500 mL each) to yield 17 fractions (F1−F17). Fraction F2 (480 mg) was purified by CC on Sephadex LH-20 with MeOH as eluent to give five subfractions (F2.1−F2.5). Fraction F2.3 was further chromatographed by CC on Sephadex LH-20 with MeOH as eluent, yielding compound **5**[29] (2.6 mg). Fraction F5 (137.8 mg) was purified by CC on Sephadex LH-20 with MeOH as eluent to give compound **3** (7.1 mg). Fraction F6 (122 mg) was subjected to CC over silica gel using hexane/EtOAc (5.5:4.5) as eluent, yielding compound **6** (19.7 mg). Fraction F7 was chromatographed by CC over silica gel using hexane/*tert*-butyl methyl ether (4.5:6.5) as eluent, giving five subfractions (F7.1−F7.5). Subfraction F7.5 (43 mg) was further purified by CC on Sephadex LH-20 with MeOH as eluent, yielding **1** (12 mg).

An aliquot (700 mg) of the aqueous layer of the MeOH extract was subjected to CC on silica gel using CH_2Cl_2/MeOH (8.5:1.5) as eluent to obtain 11 fractions (M1−M11). Fraction M6 (30 mg) was purified by Sephadex LH-20 with MeOH to give four subfractions (M6.1−M6.4). Subfractions M6.3 (12 mg) and M6.4 (18 mg) were further chromatographed over RP-HPLC using MeOH/H_2O (6:4, flow 2.5 mL/min) as eluent to yield **4** (9.5 mg) and **2** (4.2 mg), respectively.

Computational Study. A model of quercetin was built using the graphical user interface by MacroModel (Maestro GUI),[43] and a 1000 iteration step Monte Carlo simulation was carried out randomizing all rotatable bonds. Each conformation was energy minimized with the force field AMBER* united atom and the GB/SA water implicit model of salvation and deduplicated according to a root-mean-square deviation (rmsd) in the atomic coordinates and an energy difference respectively lower than 0.25 Å and 1 kcal/mol.

Following the computational work of our recent communications,[22b,33] the Protein Data Bank crystallographic models 1GOS[31] and 2BXR[32] were considered for the hMAO-A and hMAO-B docking experiments, respectively. Both PDB models were obtained as adducts with two similar compounds covalently linked to the FAD N5 nitrogen.

The pretreatment of the original PDB structures consisted of a 48 kcal/mol constrained energy minimization of those residues at a radius of 15 Å from the N5 of the isoalloxazine ring, to restore the natural planarity of the isoalloxazine FAD ring and relax the active site amino

acids. In the resulting energy minimum structures the covalent ligands were removed (clorogyline for 2BXR and pargyline for 1GOS) and used as receptor models. This was performed with the force field AMBER* united atom and the GB/SA water implicit model of solvation as implemented in MacroModel ver. 7.2.[43]

The flexible docking experiments were carried out with the same software. The starting configuration was obtained by manual positioning of the global minimum energy quercetin conformation in a configuration with the A ring close to the cofactor in a stacking interaction with MAO-A Tyr407 and Tyr444 and MAO-B Tyr398 and Tyr495. Using the MOLS directive for roto-translational settings, each ligand was allowed to rotate fully ±180°, and the translational tolerance was fixed at ±10 Å. One thousand MC iterations were carried out, and each configuration was energy minimized with AMBER* united atom and the GB/SA water implicit model of solvation. During the MC search the same constrained energy minimization conditions were adopted. The obtained MC ensemble was then fully energy minimized removing any constant force.

The interaction energy of all complexes before and after full relaxation was computed according to the MOLINE method[34] and converted to average state equations.

All calculations were performed by a Linux cluster of five Intel Xeon dual processors at 3.2 GHz with 2 Gb of RAM. Graphic manipulations and analysis of the docking experiments were performed by the Maestro GUI ver 4.1.012.[43] Ligplot ver 4.0[44] and Pymol ver 0.98[45] were used to create Figures 2 and 3.

Biochemistry. All chemicals were commercial reagents of analytical grade and were used without purification. Bovine brain mitochondria were isolated using previously described methods.[46] In all experiments the MAO activities of the beef brain mitochondria were determined by a fluorimetric method[47] using kinuramine as a substrate at four different concentrations ranging from 5 μM to 0.1 mM. The incubation mixtures contained 0.1 mL of 0.25 M K_3PO_4 buffer (pH 7.4), mitochondria (6 mg/mL), and drug solutions with concentrations ranging from 0 to 10^{-3} μM.

The solutions were incubated at 38 °C for 30 min. Addition of $HClO_4$ ended the reaction. The samples were centrifuged at 10000*g* for 5 min, and the supernatant was added to 2.7 mL of 0.1 N NaOH. The MeOH extract was dissolved in DMSO and 10, 30, and 50 μL of the resulting solution added to the reaction mixture. Quercetin was dissolved in DMSO and added to the reaction mixture from 0 to 10^{-3} μM. To study the inhibition of quercetin on the activities of both MAO-A and -B separately, the mitochondrial fractions were preincubated at 38 °C for 30 min before adding the specific inhibitors (0.5 μM L-deprenyl to estimate MAO-A activity and 0.05 μM clorgyline to assay the isoform B), considering that MAO-A is irreversibly inhibited by a low concentration of clorgyline, but is unaffected by a low concentration of L-deprenyl, which is used in the MAO-B form. Fluorimetric measurements were recorded with a Perkin-Elmer LS 50B spectrofluorimeter. The protein concentrations were then determined.[48] Resulting data are the means of three or more experiments performed in duplicate.

In Vivo Analysis. All procedures were carried out in accordance with the guidelines of the Council of European Communities and the approval of the Bioethical Committee of the Italian National Institute of Health. Male CD1 mice (Arlan, Italy), weighing 25−28 g, were housed in a central facility and maintained under controlled conditions of humidity and temperature (22 ± 1 °C), with food and water ad libitum and standard alternating 12-h periods of light and darkness, until behavioral testing.

Quercetin isolated from *H. hircinum* was suspended in carboxymethylcellulose sodium salt (CMC) (0.5%) and injected intraperitoneally (1, 10, or 100 mg/kg) in a constant volume of 0.01 mL/g of body weight 60 min before the FST session.

Forced Swimming Test Procedure. The forced swimming test (FST) procedure was performed according to the original two-day procedure.[23] Between 9 and 12 a.m. of the first day (pretest session), mice, from the naïve control group, the CMC vehicle, and the quercetin-treated groups ($n = 10$ per group), were individually plunged in a glass cylinder (height 33 cm × diameter 20 cm) containing 15 cm of tap water at a constant temperature of 25 °C and placed inside a sound-attenuated room, where a 100-W lamp was the only source of illumination. The animals were left to swim in the clean water for 15 min before being removed, gently dried with a towel, and returned to their home cage. Twenty-four hours later (test session day), the naïve controls and the treated groups were re-exposed to the tank for 10 min, 60 min after an ip injection of either vehicle (CMC) or quercetin doses, and the session was videotaped using a DV-C videocassette recorder (Sony). The animals' behavior during the first 5-min period of FST was later scored as activity (presence of movement) and immobility (absence of movement). A mouse was judged to be immobile when it remained floating in the water and only made the movements necessary to keep its head above the water.[23]

Statistical Analysis. Comparisons between naïve controls, vehicle CMC group, and quercetin-treated groups were performed by two-way analysis of variance (ANOVA), to analyze differences in the immobility time scored in FST, followed by a multiple Duncan test for comparison between group differences, with $p < 0.05$ considered statistically significant.

Acknowledgment. This work was supported by grants from MURST. We also acknowledge Mr. Anton Gerada, a professional translator, Fellow of the Institute of Translation and Interpreting of London and Member of AIIC (Association Internationale des Interprêtes de Conférences, Geneva), for revising the manuscript.

Supporting Information Available: Molecular modeling thermodynamic results and interactions of quercetin with each residue within MAO isoforms. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Russo, N.; Toscano, M.; Uccella, N. *J. Agric. Food Chem.* **2000**, *48*, 3232−3237.
(2) Zhang J.; Brodbelt J. S. *J. Am. Chem. Soc*. **2004**, *126*, 5906−5919.
(3) Sicheri, F.; Moarefi, I.; Kuriyan, J. *Nature* **1997**, *385*, 602−609.
(4) Walker, E. H.; Pacold, M. E.; Perisic, O.; Stephens, L.; Hawkins, P. T.; Whymann, M. P.; Williams, R. L. *Mol. Cell* **2000**, *6*, 909−916.
(5) Steiner, R. A.; Kalk, K. H.; Dijkstra, B. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16625−16630.
(6) Butterweck, V.; Jurgenliemk, G.; Nahrstedt, A.; Winterhoff, H. *Planta Med.* **2000**, *66*, 3−6.
(7) Patil C. S.; Singh V. P.; Satyanarayan P. S.; Jain N. K.; Singh A.; Kulkarni S. K. *Pharmacology* **2003**, *69* (2), 59−67.
(8) Singh, A.; Naidu, P. S.; Kulkarni, S. K. *Pharmacology* **2003**, *68* (2), 81−88.
(9) Burda, S.; Oleszek, W. *J. Agric. Food Chem*. **2001**, *49* (6), 2774−2779.
(10) Anjaneyulu, M.; Chopra, K.; Kaur, I. *J. Med. Food* **2003**, *6* (4), 391−395.
(11) Thiede, H. M.; Walper, A. *J. Geriatr. Psychiatry Neurol.* **1994**, *7*, 54−56.
(12) Morand, C.; Manach, C.; Craspy, V.; Remesy, C. *Biofactors* **2000**, *12* (1−4), 169−174.
(13) Rivera, F.; Urbanavicius, J.; Gervaz, E.; Morquio, A.; Dajas F. *Neurotox. Res*. **2004**, *6* (7−8), 543−553.
(14) Pistelli, L.; Bertoli, A.; Zucconelli, S.; Morelli, I.; Panizzi, L.; Menichini, F. *Fitoterapia* **2000**, *71*, 138−140.
(15) Dar, A.; Khatoon, S. *Pharmacol. Biochem. Behav.* **2000**, *65* (1), 1−6.
(16) Wagner, H.; Bladt, S. *J. Geriar. Psychiatry Neurol.* **1994**, *7* (1), 65−68.
(17) Bhattacharya, S.; Chakrabarti, A.; Chatterjee, S. *Pharmacopsychiatry* **1998**, *31*, 22−29.
(18) Chatterjee, S.; Bhattacharya, S. K.; Wonnemann, M.; Singer, A.; Muller, W. E. *Life Sci.* **1998**, *63*, 499−510.
(19) Rocha, L.; Marston, A.; Kaplan, M. A. C.; Stoeckli-Evans, H.; Thull, U.; Testa, B.; Hostettmann, K. *Phytochemistry* **1994**, *6*, 1381−1385.
(20) Gnerre, C.; von Poser, G. L.; Ferraz, A.; Viana, A.; Testa, B.; Rates, S. M. K. *J. Pharm. Pharmacol.* **2001**, *53*, 1273−1279.
(21) Pieroni, A.; Quave, C. L.; Santoro, R. F. *J. Ethnopharmacol.* **2004**, *95*, 373−384.
(22) (a) Chimenti, F.; Bolasco, A.; Manna, F.; Secci, D.; Chimenti, P.; Befani, O.; Turini, P.; Giovannini, V.; Mondovi', B.; Cirilli, R.; La Torre, F. *J. Med. Chem.* **2004**, *47* (8), 2071−2074. (b) Chimenti, F.; Secci, D.; Bolasco, A., Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F. *Bioorg., Med. Chem. Lett*. **2004**, *14/14*, 3697−3703.
(23) Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. *Eur. J. Pharmacol.* **1978**, *47* (4), 379−391.
(24) Graalfs, H.; Fröhlich, R.; Wolff, C.; Mattay, J. *Eur. J. Org. Chem.* **1999**, *5*, 1057−1073.
(25) Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier: Amsterdam, 1989.
(26) Alluis, B.; Dangles, O. *Helv. Chim. Acta* **2001**, *84*, 1133−1156.
(27) Encarnación, D. R.; Noguerias, C. L.; Salinas, V. H. A.; Anthoni, U.; Nielsen, P. H.; Christophersen, C. *Acta Chem. Scand*. **1999**, *53*, 375−377.

(28) Chen, M. T.; Kuoh, Y. P.; Wang, C. H.; Chen, C. M.; Kuoh, C. S. *J. Chin. Chem. Soc.* **1989**, *36*, 165−168.
(29) Data of 1,6-dihydroxy-5,7-dimethoxyxanthone (**5**): yellow solid; mp 221−222 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.71 (1H, dd, J = 8, 8.4, H-3), 7.51 (1H, s, H-8), 7.11 (1H, d, J = 8.4, H-4), 6.84 (1H, d, J = 8, H-2), 4.11 (3H, s, OCH_3), 4.07 (3H, s, OCH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 182 (C-9), 163.1 (C-1), 157 (C-4a), 148.5 (C-6), 147.9 (C-7), 137.1 (C-3), 136.5 (C-5), 134.4 (C-5a), 118 (C-8a), 110.9 (C-2), 109 (C-9a), 108.5 (C-4), 100.4 (C-8), 62 (OCH_3), 56.5 (OCH_3).
(30) Cesura, A. M.; Pletscher, A. *Prog. Drug Res.* **1992,** *38*, 171−297.
(31) Binda, C.; Newton-Vinson, P.; Hubalek, F.; Edmondson, D. E.; Mattevi, A. *Nat. Struct. Biol.* **2002**, *9*, 22−26. Data deposition: www.pdb.org (PDB ID code 1GOS).
(32) De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12684−12689. Data deposition: www.pdb.org (PDB ID code 2BXR, 2BXS, and 2BYB).
(33) Chimenti, F.; Maccioni, E.; Secci, D.; Bolasco, A., Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F.; Cirilli; R.; La Torre, F.; Cardia, M. C.; Distinto, S. *J. Med. Chem.* **2006**, *48*, 7113−7122.
(34) Alcaro, S.; Gasparrini, F.; Incani, O.; Mecucci, S.; Misiti, D.; Pierini, M.; Villani, C. *J. Comput. Chem.* **2000**, *21*, 515−530.
(35) Abell, C. W.; Kwan, S. W. *Prog. Nucleic Acid Res. Mol. Biol.* **2000**, *65*, 129−156.
(36) Wouters, J. *Curr. Med. Chem.* **1998**, *5* (2), 137−162.
(37) Thiede, H. M.; Walper, A. *J. Geriatr. Psychiatry Neurol.* **1994**, *7*, 54−56.
(38) Lee, M. H.; Lin, R. D.; Shen, L. Y.; Yang, L. L.; Yen, K. Y.; Hou, W. C. *J. Agric. Food Chem.* **2001**, *49* (11), 5551−5555.
(39) Miura, H.; Naoi, M.; Nakahara, D.; Ohta, T.; Nagatsu, T. *Pharmacol. Biochem. Behav.* **1996**, *53* (2), 469−475.
(40) Picq, M.; Cheav, S. L.; Prigent, A. F. *Life Sci.* **1991**, *49* (26), 1979−1988.
(41) Goutman, J. D.; Waxemberg, M. D.; Donate-Oliver, F.; Pomata, P. E.; Calvo, D. J. *Eur. J. Pharmacol.* **2003**, *461* (2−3), 79−87.
(42) Goutman, J. D.; Calvo, D. J. *Br. J. Pharmacol.* **2004**, *141* (4), 717−727.
(43) MacroModel ver 7.2, Schrödinger Inc.: Portland, OR, 1998−2001. (b) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.
(44) Wallace, A. C.; Laskowski, R. A.; Thornton, J. *Protein Eng.* **1995**, *8*, 127−134.
(45) DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientific: San Carlos, CA, 2002; http://www.pymol.org.
(46) Basford, R. E. *Methods Enzymol.* **1967**, *10*, 96−101.
(47) Matsumoto, T.; Suzuki, O.; Furuta T.; Asai, M.; Kurokawa, Y.; Rimura, Y.; Katsumata, Y.; Takahashi, I. *Clin. Biochem.* **1985**, *18*, 126−129.
(48) Bradford. M. M. *Anal. Biochem.* **1976**, *72*, 248−254.

NP060015W