Full Paper

Synthesis and Evaluation of Human Monoamine Oxidase Inhibitory Activities of Some 3,5-Diaryl-*N*-substituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamide Derivatives

Kerem Şentürk[1], Oya Unsal Tan[1], Samiye Yabanoğlu Çiftçi[2], Gülberk Uçar[2], and Erhan Palaska[1]

[1] Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Hacettepe University, Ankara, Turkey
[2] Faculty of Pharmacy, Department of Biochemistry, Hacettepe University, Ankara, Turkey

Sixteen 3-aryl-5-(4-fluorophenyl)-*N*-substituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives were synthesized and their structure were identified by UV, IR, ^{1}H NMR, mass spectra, and microanalyses. The compounds were evaluated *in vitro* for their human monoamine oxidase (hMAO) inhibitory activities and their MAO-A and -B selectivity. All the compounds were found to potently inhibit MAO-A isoforms. 5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-*N*-methyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (1.0×10^{-3} μM) was found to inhibit hMAO-A most selectively and potently. The binding mode of 5-(4-fluorophenyl)-3-(4-methoxyphenyl)-*N*-methyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide to hMAO-A was also predicted using docking studies.

Keywords: 2-Pyrazoline / 4,5-Dihydropyrazole / Docking / Monoamine oxidase

Received: December 8, 2011; Revised: April 18, 2012; Accepted: April 25, 2012

DOI 10.1002/ardp.201100448

Introduction

The monoamine oxidase (MAO) catalyzes the oxidative deamination of biogenic amines to aldehydes with radicalic and polar nucleophilic mechanisms. MAO exists in two isoforms, MAO-A and MAO-B. These isoforms are encoded by two different genes and distinguished by different substrate specificities and sensivities to the selective inhibitors. MAO-A deaminates seratonin and norephinephrine [1–5]. Reversible inhibition of MAO-A in the brain causes increased brain levels of transmitters such as dopamine and serotonin. Selective MAO-A inhibitors, such as clorgyline and moclobemide, are useful in the treatment of depression. MAO-B selectively catalyses the oxidation of 2-phenylethylamine and benzylamine. The abnormal MAO-B activity is implicated in neurodegenerative disorders and the selective MAO-B inhibitor l-deprenyl (selegiline) is widely used in the treatment of Alzheimer's and Parkinson's disease [4–9].

Adverse side effects of non-selective and irreversible MAO inhibitors such as iproniazid, isocarboxazid, and phenelzine have numerous, significant interactions with dietary sympathomimetic amines such as tyramine and cause hypertensive crises, so-called ''cheese effect'', blurred vision, and dizziness. They also potentiate the effects of alcohol and antihistamines, tricyclic antidepressants, tranquilizers, some pain relievers, and muscle relaxants. In the last decades, efforts have been directed to the design, synthesis, and study of new MAO inhibitors which display low toxicity and relative selectivity for the treatment of various psychiatric and neurodegenerative disorders. These inhibitors belong to various chemical classes and show different modes of enzyme inhibition resulting from the covalent mechanism-based interaction of the compounds (clorgyline, selegiline, and rasagiline) to non-covalent interaction-based ones (brofaromine, toloxatone, and moclobemide) with MAO isoforms. Despite considerable progress in understanding the interactions of the two enzyme forms with their preferred substrates and inhibitors, no general rules are yet available for the rational design of potent, reversible, and selective inhibitors of MAO, possibly due the fact that the mechanism of interaction of the new drugs with MAO isoforms have not been fully characterized.

During recent years there has been an increasing amount of work done on 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives, many of which were found to possess selective MAO inhibitory properties. 1,3,5-Triphenyl-, 1-acyl-, and 3,5-diaryl-

Correspondence: Dr. Erhan Palaska, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Hacettepe, 06100 Ankara, Turkey.
E-mail: epalaska@hacettepe.edu.tr
Fax: +90-3123051872

4,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives showed remarkable MAO inhibitory [10–14] and antidepressant [11, 15, 16] activities. The substitution of N1 in the 4,5-dihydro-1*H*-pyrazole nucleus with the carbothioamide group favored the inhibitory activity toward the MAOs [11, 13, 14, 17]. Also, in our previous study in which we evaluated MAO inhibitory activities of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives having a carbothioamide substituent at the N1 position, we observed that the compounds mostly had selective inhibitory activity against the MAO-A enzyme [18].

Pursuing our studies on MAO inhibitor compounds, in the present work, we synthesized 3-aryl-5-(4-fluorophenyl)-*N*-substituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives **1–16** and assayed their MAO-A and MAO-B inhibitory activities by *in vitro* tests. Docking studies for the most potent and selective derivative were performed in order to gain more insight toward its binding mode.

Results

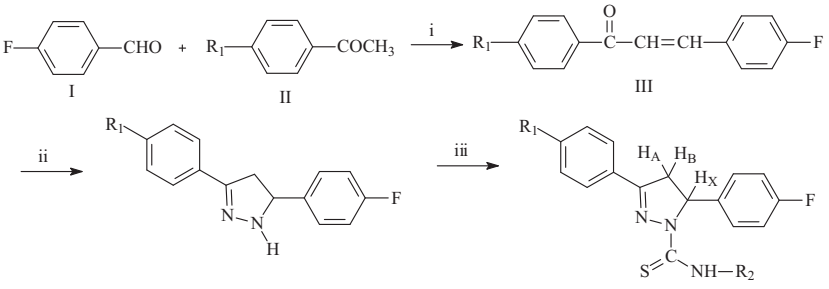
The synthetic route used to synthesize **1–16** is outlined in Scheme 1. The starting compounds, 1-aryl-3-(4-fluorophenyl)-2-propen-1-ones **III**, were synthesized by condensing 4-fluorobenzaldehyde **I** with acetophenones **II** according to the previously reported Claisen–Schmidt condensation reaction procedures [19, 20]. The *N*-substituted carbothioamide derivatives **1–16** were synthesized by the reaction of appropriate 1-aryl-3-(4-fluorophenyl)-2-propen-1-ones **III** and hydrazine hydrate followed by the addition of isothiocyanates. Although the syntheses of **5** and **9** in the series have been reported previously [21], they were also synthesized in the present study to investigate their human monoamine oxidase (hMAO) inhibitory activities.

The potential inhibitory actions of novel compounds on hMAO activity were investigated by measuring their effects on the production of hydrogen peroxide from *p*-tyramine, using the Amplex-Red MAO assay kit and hMAO isoforms in microsomes prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for hMAO-A or hMAO-B. The inhibition of hMAO activity was evaluated using a fluorimetric method. The synthesized derivatives and reference inhibitors (selegiline and moclobemide) were unable to react directly with Amplex-Red reagent. The compounds did not react with resorufin used in the method and did not quench the fluorescence generated by this product.

The hMAO-A and hMAO-B inhibition data are reported in Table 1 together with the selectivity index (SI). According to the enzymatic assays, all the synthesized compounds were found to be potent (K_i: 1×10^{-3}–1.16 μM) and selective (SI: 4.65×10^{-4}–0.89) MAO-A inhibitors. The inhibition profile was also found to be competitive and reversible for all compounds.

Among them, 5-(4-fluorophenyl)-3-(4-methoxyphenyl)-*N*-methyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide **4** is the most effective (K_i: 1.0×10^{-3} μM) compound in this series. The SI of **4** toward hMAO-A was calculated as 4.65×10^{-4}, which is smaller than that of moclobemide.

As observed from the data, the introduction of a 4-methoxy group on the 3-phenyl ring increased the MAO-A inhibitory activity (K_i: 1.0×10^{-3}, 0.21, 0.24, and 0.12 μM for **4**, **8**, **12**, and **16**, respectively) and selectivity (SI: 4.65×10^{-4}, 0.11, 0.15, and 0.13 for **4**, **8**, **12**, and **16**, respectively) of the derivatives. In addition, the substitution by a chlorine atom on the phenyl ring (except **2**) produced comparable hMAO-A inhibitory activity to the substitution by a methoxy group. However, the inhibitory potencies of 3-phenyl (**1**, **5**, **9**, and **13**) and 3-(4-methylphenyl) derivatives (**3**, **7**, **11**, and **15**) were found to be lower than those of the other derivatives.



1:R_1=H, R_2= CH_3; **2**:R_1=Cl, R_2= CH_3; **3**:R_1=CH_3, R_2= CH_3; **4**:R_1=CH_3O, R_2= CH_3; **5**: R_1=H, R_2= C_2H_5; **6**:R_1=Cl, R_2= C_2H_5; **7**:R_1=CH_3, R_2= C_2H_5; **8**:R_1=CH_3O, R_2= C_2H_5; **9**:R_1=H, R_2= C_6H_5; **10**:R_1=Cl, R_2= C_6H_5; **11**:R_1=CH_3, R_2= C_6H_5; **12**:R_1=CH_3O, R_2= C_6H_5; **13**:R_1=H, R_2= C_3H_5; **14**:R_1=Cl, R_2= C_3H_5; **15**:R_1=CH_3, R_2= C_3H_5; **16**:R_1=CH_3O, R_2= C_3H_5;

Scheme 1. Synthesis of the compounds. (i) KOH, 10°C, 1 h, (ii) H_2NNH_2, reflux, 2 h, and (iii) R_2-NCS, rt, 3 h.

Table 1. Experimental K_i values corresponding to the inhibition of hMAO isoforms by 1-*N*-substituted thiocarbamoyl-3-aryl-5-(4-fluorophenyl)-4,5-dihydro-(1*H*)-pyrazole derivatives

Comp	R_1	R_2	K_i value for MAO-A (μM)	K_i value for MAO-B (μM)	SI[a)]	MAO selectivity, inhibition type
1	H–	CH_3–	$0.75 \pm 1.0 \times 10^{-3}$	1.20 ± 0.11	0.63	MAO-A, competitive and reversible
2	Cl–	CH_3–	0.55 ± 0.02	2.20 ± 0.13	0.25	MAO-A, competitive and reversible
3	CH_3–	CH_3–	0.60 ± 0.03	1.30 ± 0.08	0.46	MAO-A, competitive and reversible
4	CH_3O–	CH_3–	$1.0 \times 10^{-3} \pm 1.0 \times 10^{-4}$	2.15 ± 0.11	4.65×10^{-4}	MAO-A, competitive and reversible
5	H–	C_2H_5–	1.16 ± 0.08	1.30 ± 0.14	0.89	MAO-A, competitive and reversible
6	Cl–	C_2H_5–	0.10 ± 0.07	0.20 ± 0.01	0.50	MAO-A, competitive and reversible
7	CH_3–	C_2H_5–	0.33 ± 0.01	0.85 ± 0.06	0.39	MAO-A, competitive and reversible
8	CH_3O–	C_2H_5–	0.21 ± 0.01	1.90 ± 0.05	0.11	MAO-A, competitive and reversible
9	H–	C_6H_5–	0.78 ± 0.05	1.10 ± 0.09	0.71	MAO-A, competitive and reversible
10	Cl–	C_6H_5–	0.21 ± 0.01	0.70 ± 0.05	0.30	MAO-A, competitive and reversible
11	CH_3–	C_6H_5–	0.35 ± 0.01	0.80 ± 0.03	0.44	MAO-A, competitive and reversible
12	CH_3O–	C_6H_5–	0.24 ± 0.02	1.58 ± 0.11	0.15	MAO-A, competitive and reversible
13	H–	C_3H_5–	0.61 ± 0.04	1.50 ± 0.12	0.66	MAO-A, competitive and reversible
14	Cl–	C_3H_5–	0.12 ± 0.01	0.23 ± 0.01	0.52	MAO-A, competitive and reversible
15	CH_3–	C_3H_5–	0.80 ± 0.04	1.02 ± 0.08	0.78	MAO-A, competitive And reversible
16	CH_3O–	C_3H_5–	0.12 ± 0.01	0.95 ± 0.03	0.13	MAO-A, competitive and reversible
Selegiline			9.06 ± 0.44	$0.09 \pm 4.0 \times 10^{-3}$	99.92	MAO-B, competitive and irreversible
Moclobemide			$5 \times 10^{-3} \pm 1.3 \times 10^{-4}$	1.22 ± 0.08	4×10^{-3}	MAO-A, competitive and reversible

K_i values were determined from the kinetic experiments in which *p*-tyramine (substrate) was used at 500 μM to measure MAO-A and at 2.5 mM to measure MAO-B. Pargyline or clorgyline were added at 0.50 μM to determine the isoforms A and B. Newly synthesized compounds and the known inhibitors were preincubated with the homoganates for 60 min at 37°C. Each value represents the mean ± SEM of three independent experiments. K_i values corresponding to the inhibition of MAO isoforms by moclobemide as selective MAO-A inhibitor, and selegiline as selective MAO-B inhibitor, were also determined to assess the inhibitory potencies of the novel compounds.

[a)] Selectivity index. It was calculated as K_i (MAO-A)/K_i (MAO-B).

It was also observed that the presence of groups smaller than methoxy (H, Cl, and CH_3) on the phenyl ring caused a noticeable decrease in selectivity toward MAO-A.

On the other hand, when the substitution of carbothioamide group is concerned, it can be said that smaller groups are preferable for the activity of MAO-A. The introduction of a bulky group on the carbothioamide moiety did not make any contribution to the activity.

The docking study was performed by using MOE software to understand the orientations of the most active ligand in the MAO-A enzyme. 2Z5X, a high resolution crystallographic structure, was used as receptor model after correcting the FAD double bonds. Since the compounds were racemic mixtures we constructed the docking studies with two enantiomers of the ligand.

Because **4** was the most active compound on MAO-A, we mainly considered about its orientations. For the *S* enantiomer, it was observed that the 4-methoxyphenyl ring at position 3 was oriented close to the hydrophobic cavity obtained by Tyr407 and 444 while the 4-fluorophenyl ring at position 5 was face to face with Phe208. The oxygen of the methoxy group interacted with the OH group of Tyr444 (<3 Å). In addition to these, the N–H proton of the thiocarbamoyl moiety approached the C=O of Thr336 (<4 Å; Fig. 1). For the *R* enantiomer, it was observed that the 4-fluorophenyl ring at position 5 was oriented close to the hydrophobic cavity obtained by Tyr407 and Tyr444 instead of the 4-methoxyphenyl ring at position 3 in the *S* enantiomer. The *R* enantiomer did not make any favorable interaction with the enzyme. Considering their orientations and S scores (data not shown) we infered that the *S* enantiomer of **4** was more suitable in structure for the MAO-A enzyme than the *R* enantiomer. In order to visualize the selectivity of **4**, the structure of MAO-A docked with the *S* enantiomer of **4** was superimposed with MAO-B. In Fig. 2, it is seen that Tyr326, known as an amino acid responsible for selectivity [22], clashed with the ligand.

Discussion

In this study, a series of new 3-aryl-5-(4-fluorophenyl)-*N*-substituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives was synthesized in order to evaluate their inhibitory activities on hMAO-A and hMAO-B enzymes. The binding mode of the most potent compound inside the MAO-A active site was predicted using a docking technique.

Considering the inhibitory activities and docking studies of the compounds together, it can be presumed that: (i) the 3-

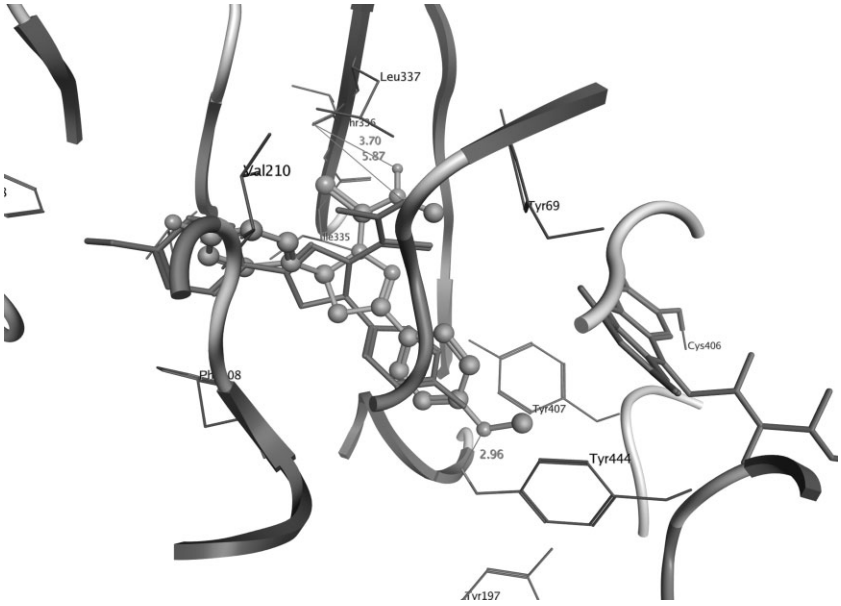


Figure 1. The orientation of *S* (ball and stick) and *R* (stick) enantiomers of **4** in MAO-A active pocket.

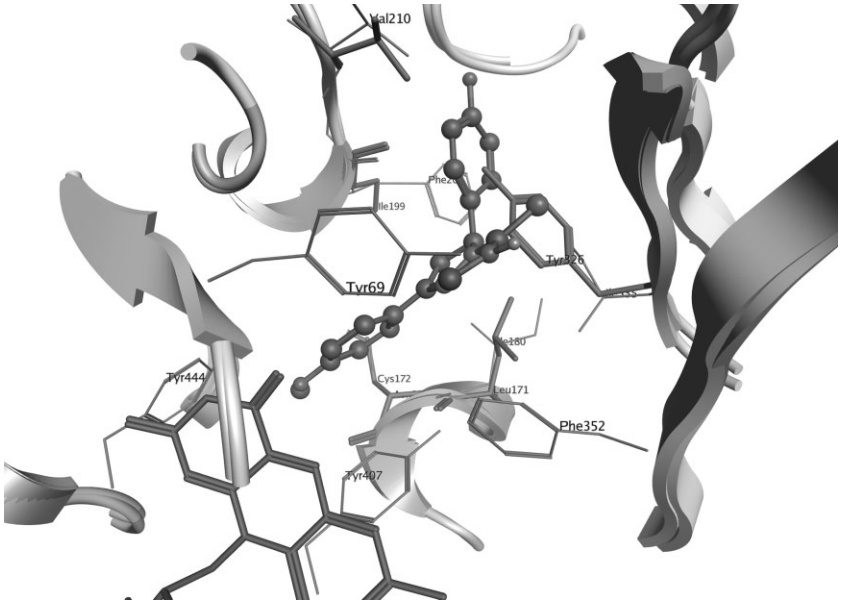


Figure 2. Superimposition of MAO-A (thin) docked with the *S* (ball and stick) enantiomer of **4** and MAO-B (stick).

aryl-5-(4-fluorophenyl)-*N*-substituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamide structure is a suitable scaffold for the MAO-A enzyme; (ii) the presence of a hydrogen bond acceptor group such as methoxy or chloro on the 3-phenyl ring increases the potency and selectivity toward MAO-A; (iii) substituents bulkier than methyl on the carbothioamide moiety do not make any contribution to the selectivity; and (iv) the synthesized pyrazole derivatives may be promising candidates as selective and potent antidepressant agents since **4** in this series appeared as a more selective novel MAO-A inhibitor than moclobemide, the known potent MAO-A inhibitor.

Experimental

Chemistry

All chemicals were purchased from Fluka, E. Merck, and Aldrich Chemical Company. Melting points were determined with a Thomas-Hoover capillary melting point apparatus

(Philadelphia, PA, USA) and are uncorrected. IR spectra were recorded on a Perkin Elmer FTIR system, Spectrum BX IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 FT NMR spectrophotometer using TMS as an internal reference (chemical shift represented in δ ppm). The ESI-MS spectra were measured on a Micromass ZQ MS spectrometer (MassLynx 4.1 software). Elemental analyses (C, H, N) were performed on a Leco CHNS 932 analyzer.

*Preparation of 3-aryl-5-(4-fluorophenyl)-N-substituted-4,5-dihydro-1H-pyrazole-1-carbothioamide derivatives (**1**–**16**)*

A solution of appropriate 1-aryl-3-(4-fluorophenyl)-2-propen-1-one (10 mmol) and hydrazine hydrate (20 mmol) was refluxed in ethanol for 2 h. The reaction mixture was cooled to −18°C, and the solid mass separated out was filtered off and dissolved in dry diethyl ether. Substituted isothiocyanate (10 mmol) and three drops of triethylamine were added and stirred for 3 h at room temperature. The mixture was evaporated to dryness and the residue was crystallized from suitable solvents.

*5-(4-Fluorophenyl)-N-methyl-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**1**)*

Yield: 28.4%, mp 151–153°C. IR: (cm^{-1}): 3363 (N–H), 1605 (C=N), 1397 (C^4–H), 1347 (C=S), 1105 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.95 (3H; d; NH–C$\underline{H}_3$), 3.11 (1H; dd; H$_A$-pyrazole-H$_4$), 3.86 (1H; dd; H$_B$-pyrazole-H$_4$), 5.91 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18.4, J_{AX}: 3.6, J_{BX}: 11.2 Hz), 7.07–7.87 (9H; m; aromatic prot.), 8.54 (1H; q; CS–N$\underline{H}$–CH$_3$). ESI-MS (*m*/*z*); 336 [M+Na]$^+$ (100%), 314 [M+H]$^+$. Anal. Calcd. for $C_{17}H_{16}FN_3S$: C, 65.15; H, 5.15; N, 13.41; S, 10.23. Found: C, 64.28; H, 5.16; N, 13.20; S, 10.44.

*3-(4-Chlorophenyl)-5-(4-fluorophenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2**)*

Yield: 37.8%, mp 138–139°C. IR: (cm^{-1}): 3271 (N–H), 1601 (C=N), 1388 (C^4–H), 1356 (C=S), 1089 (C^5–N^1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.96 (3H; d; NH–C$\underline{H}_3$), 3.14 (1H; dd; H$_A$-pyrazole-H$_4$), 3.87 (1H; dd; H$_B$-pyrazole-H$_4$), 5.92 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18.4, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 7.10–7.92 (8H; m; aromatic prot.), 8.62 (1H; q; CS–N$\underline{H}$–CH$_3$). ESI-MS (*m*/*z*); 372 [M+Na+2]$^+$, 370 [M+Na]$^+$ (100%), 348 [M+H]$^+$. Anal. Calcd. for $C_{17}H_{15}ClFN_3S$: C, 58.70; H, 4.35; N, 12.08; S, 9.22. Found: C, 58.16; H, 4.42; N, 11.97; S, 9.26.

*5-(4-Fluorophenyl)-N-methyl-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**3**)*

Yield: 13.3%, mp 125–127°C. IR: (cm^{-1}): 3276 (N–H), 1603 (C=N), 1392 (C^4–H), 1317 (C=S), 1108 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.35 (3H; s; Ar–CH$_3$), 2.97 (3H; d; NH–C$\underline{H}_3$), 3.11 (1H; dd; H$_A$-pyrazole-H$_4$), 3.85 (1H; dd; H$_B$-pyrazole-H$_4$), 5.91 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 7.10–7.78 (8H; m; aromatic prot.), 8.51 (1H; q; CS–N$\underline{H}$–CH$_3$). ESI-MS (*m*/*z*); 350 [M+Na]$^+$ (100%), 328 [M+H]$^+$. Anal. Calcd. for $C_{18}H_{18}FN_3S$: C, 66.03; H, 5.54; N, 12.83; S, 9.79. Found: C, 66.01; H, 5.59; N, 12.92; S, 9.84.

*5-(4-Fluorophenyl)-3-(4-methoxylphenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**4**)*

Yield: 42.4%, mp 128–129°C. IR: (cm^{-1}): 3362 (N–H), 1605 (C=N), 1398 (C^4–H), 1344 (C=S), 1034 (C^5–N^1). ^{1}H NMR (acetone-d_6, 400 MHz): δ 3.08 (3H; d; NH–C$\underline{H}_3$), 3.17 (1H; dd; H$_A$-pyrazole-H$_4$), 3.85 (3H; s; OCH$_3$), 3.92 (1H; dd; H$_B$-pyrazole-H$_4$), 6.01 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18, J_{AX}: 4.0, J_{BX}: 11.2 Hz), 6.98–7.82 (8H; m; aromatic prot.), 8.16 (1H; q; CS–N$\underline{H}$–CH$_3$). ESI-MS (*m*/*z*); 366 [M+Na]$^+$ (100%), 344 [M+H]$^+$. Anal. Calcd. for $C_{18}H_{18}FN_3OS$: C, 62.95; H, 5.28; N, 12.24; S, 9.34. Found: C, 62.84; H, 5.18; N, 12.16; S, 9.23.

*N-Ethyl-5-(4-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**5**)*

Yield: 41.0%, mp 83–84°C. IR: (cm^{-1}): 3345 (N–H), 1601 (C=N), 1377 (C^4–H), 1316 (C=S), 1036 (C^5–N^1). ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.20 (3H; t; CH$_2$–C$\underline{H}_3$) 3.20 (1H; dd; H$_A$-pyrazole-H$_4$), 3.65 (2H; m; NH–C$\underline{H}_2$–CH$_3$), 3.97 (1H; dd; H$_B$-pyrazole-H$_4$), 6.04 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18, J_{AX}: 4.0, J_{BX}: 11.6 Hz), 7.03–7.87 (9H; m; aromatic prot.), 8.29 (1H; s; CS–N$\underline{H}$–C$_2$H$_5$). ESI-MS (*m*/*z*); 350 [M+Na]$^+$ (100%), 328 [M+H]$^+$. Anal. Calcd. for $C_{18}H_{18}FN_3S$: C, 66.03; H, 5.54; N, 12.83; S, 9.79. Found: C, 66.29; H, 5.36; N, 12.79; S, 9.39.

*3-(4-Chlorophenyl)-N-ethyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6**)*

Yield: 27.7%, mp 123–124°C. IR: (cm^{-1}): 3363 (N–H), 1605 (C=N), 1374 (C^4–H), 1333 (C=S), 1087 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.11 (3H; t; CH$_2$–C$\underline{H}_3$) 3.09 (1H; dd; H$_A$-pyrazole-H$_4$), 3.53 (2H; m; NH–C$\underline{H}_2$–CH$_3$), 3.83 (1H; dd; H$_B$-pyrazole-H$_4$), 5.92 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18, J_{AX}: 4, J_{BX}: 11.6 Hz), 7.07–7.89 (8H; m; aromatic prot.), 8.29 (1H; s; CS–N$\underline{H}$–C$_2$H$_5$). ^{13}C NMR (DMSO-d_6): δ 175.79, 161.77 (d, J = 240.8 Hz), 153.87, 139.99 (d, J = 3.1 Hz), 135.76, 130.58, 129.48, 129.43, 128.14 (d, J = 8.4 Hz), 115.87 (d, J = 21.3 Hz), 63.43, 42.36, 40.19, 15.19. ESI-MS (*m*/*z*); 386 [M+Na+2]$^+$, 384 [M+Na]$^+$ (100%), 362 [M+H]$^+$. Anal. Calcd. for $C_{18}H_{17}ClFN_3S$: C, 59.74; H, 4.74; N, 11.61; S, 8.86. Found: C, 59.42; H, 4.67; N, 11.67; S, 9.01.

*N-Ethyl-5-(4-fluorophenyl)-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**7**)*

Yield: 33.9%, mp 121–122°C. IR: (cm^{-1}): 3372 (N–H), 1603 (C=N), 1374 (C^4–H), 1366 (C=S), 1094 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.10 (3H; t; CH$_2$–C$\underline{H}_3$), 2.32 (3H; s; Ar–C$\underline{H}_3$), 3.07 (1H; dd; H$_A$-pyrazole-H$_4$), 3.50 (2H; m; NH–C$\underline{H}_2$–CH$_3$), 3.82 (1H; dd; H$_B$-pyrazole-H$_4$), 5.89 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 7.07–7.77 (8H; m; aromatic prot.), 8.49 (1H; s; CS–N$\underline{H}$–C$_2$H$_5$). ESI-MS (*m*/*z*); 364 [M+Na]$^+$ (100%), 342 [M+H]$^+$. Anal. Calcd. for $C_{19}H_{20}FN_3S$: C, 66.83; H, 5.90; N, 12.31; S, 9.39. Found: C, 66.92; H, 5.73; N, 12.29; S, 9.45.

*N-Ethyl-5-(4-fluorophenyl)-3-(4-methoxylphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**8**)*

Yield: 36.7%, mp 139°C. IR: (cm^{-1}): 3344 (N–H), 1602 (C=N), 1369 (C^4–H), 1334 (C=S), 1111 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.13 (3H; t; CH$_2$–C$\underline{H}_3$), 3.10 (1H; dd; H$_A$-pyrazole-H$_4$), 3.54 (2H; m; NH–C$\underline{H}_2$–CH$_3$), 3.81 (3H; s; OC$\underline{H}_3$), 3.84 (1H; dd; H$_B$-pyrazole-H$_4$), 5.91 (1H; dd; H$_X$-pyrazole-H$_5$) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 6.99–7.85 (8H; m; aromatic prot.), 8.46 (1H; s; CS–N$\underline{H}$–C$_2$H$_5$). ESI-MS (*m*/*z*); 380 [M+Na]$^+$ (100%), 358 [M+H]$^+$. Anal. Calcd. for $C_{19}H_{20}FN_3OS$: C, 63.84; H, 5.64; N, 11.76; S, 8.97. Found: C, 64.03; H, 5.29; N, 11.81; S, 8.96.

*5-(4-Fluorophenyl)-N,3-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**9**)*

Yield: 22.3%, mp 178–179°C. IR: (cm^{-1}): 3343 (N–H), 1603 (C=N), 1396 (C^4-H), 1334 (C=S), 1096 (C^5-N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.23 (1H; dd; H_A-pyrazole-H_4), 3.97 (1H; dd; H_B-pyrazole-H_4), 6.05 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.2 Hz), 7.14–8.01 (14H; m; aromatic prot.), 10.19 (1H; s; CS–N<u>H</u>–C_6H_5). ESI-MS (*m*/*z*); 398 $[M+Na]^+$ (100%), 376 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{18}FN_3S$: C, 70.38; H, 4.83; N, 11.19; S, 8.54. Found: C, 70.04; H, 4.84; N, 11.02; S, 8.57.

*3-(4-Chlorophenyl)-5-(4-fluorophenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**10**)*

Yield: 20.7%, mp 134–135°C. IR: (cm^{-1}): 3314 (N–H), 1594 (C=N), 1372 (C^4–H), 1319 (C=S), 1091 (C^5–N^1). ^{1}H NMR (acetone-d_6, 400 MHz): δ 3.31 (1H; dd; H_A-pyrazole-H_4), 4.08 (1H; dd; H_B-pyrazole-H_4), 6.18 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 7.06–8.01 (13H; m; aromatic prot.), 9.97 (1H; s; CS–N<u>H</u>–C_6H_5). ESI-MS (*m*/*z*); 434 $[M+Na+2]^+$, 432 $[M+Na]^+$ (100%), 410 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{17}ClFN_3S$: C, 64.46; H, 4.18; N, 10.25; S, 7.82. Found: C, 63.93; H, 4.12; N, 10.41; S, 7.82.

*5-(4-Fluorophenyl)-3-(4-methylphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**11**)*

Yield: 39.4%, mp 161–162°C. IR: (cm^{-1}): 3338 (N–H), 1603 (C=N), 1388 (C^4–H), 1334 (C=S), 1096 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (3H; s; Ar–C<u>H</u>$_3$), 3.20 (1H; dd; H_A-pyrazole-H_4), 3.94 (1H; dd; H_B-pyrazole-H_4), 6.03 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.2 Hz), 7.13–7.90 (13H; m; aromatic prot.), 10.15 (1H; s; CS–N<u>H</u>–C_6H_5). ESI-MS (*m*/*z*); 412 $[M+Na]^+$ (100%), 390 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{20}FN_3S$: C, 70.93; H, 5.18; N, 10.79; S, 8.23. Found: C, 70.56; H, 5.07; N, 10.90; S, 8.59.

*5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**12**)*

Yield: 34.7%, mp 176–177°C. IR: (cm^{-1}): 3312 (N–H), 1606 (C=N), 1391 (C^4–H), 1315 (C=S), 1114 (C^5–N^1). ^{1}H NMR (acetone-d_6, 400 MHz): δ 3.27 (1H; dd; H_A-pyrazole-H_4), 3.87 (3H; s; O<u>C</u>H_3), 4.04 (1H; dd; H_B-pyrazole-H_4), 6.14 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 18, J_{AX}: 4, J_{BX}: 11.2 Hz), 7.02–7.92 (13H; m; aromatic prot.), 9.86 (1H; s; CS–N<u>H</u>–C_6H_5). ESI-MS (*m*/*z*); 428 $[M+Na]^+$ (100%), 406 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{20}FN_3OS$: C, 68.13; H, 4.97; N, 10.36; S, 7.91. Found: C, 67.52; H, 4.87; N, 9.82; S, 7.51.

*N-Allyl-5-(4-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**13**)*

Yield: 36.2%, mp 115–116°C. IR: (cm^{-1}): 3275 (N–H), 1602 (C=N), 1370 (C^4–H), 1323 (C=S), 1123 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.11 (1H; dd; H_A-pyrazole-H_4), 3.87 (1H; dd; H_B-pyrazole-H_4), 4.16 (2H; m; CH_2), 5.06 (1H; dd; =CH_2, H_a), 5.13 (1H; dd; =CH_2, H_b) 5.85 (1H; m; –CH=, H_x) (J_{ab}: 1.6, J_{ax}: 10.0, J_{bx}: 17.2 Hz), 5.93 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 18.4, J_{AX}: 4, J_{BX}: 11.6 Hz), 7.08–7.89 (9H; m; aromatic prot.), 8.69 (1H; t; CS–N<u>H</u>–CH_2–). ESI-MS (*m*/*z*); 362 $[M+Na]^+$ (100%), 340 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{18}FN_3S$: C, 67.23; H, 5.35; N, 12.38; S, 9.45. Found: C, 67.07; H, 5.35; N,12.54; S, 9.63.

*N-Allyl-3-(4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**14**)*

Yield: 27.5%, mp 187–188°C. IR: (cm^{-1}): 3377 (N–H), 1600 (C=N), 1372 (C^4–H), 1339 (C=S), 1090 (C^5–N^1). ^{1}H NMR (acetone-d_6, 400 MHz): δ 3.23 (1H; dd; H_A-pyrazole-H_4), 4.00 (1H; dd; H_B-pyrazole-H_4), 4.28 (2H; m; CH_2), 5.08 (1H; dd; =CH_2, H_a), 5.21 (1H; dd; =CH_2, H_b) 5.96 (1H; m; –CH=, H_x) (J_{ab}: 1.6, J_{ax}: 10.0, J_{bx}: 17.2 Hz), 6.07 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 17.6, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 7.04–7.91 (8H; m; aromatic prot.), 8.39 (1H; s; CS–N<u>H</u>–CH_2–). ESI-MS (*m*/*z*); 398 $[M+Na+2]^+$, 396 $[M+Na]^+$ (100%), 374 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{17}ClFN_3S$: C, 61.04; H, 4.58; N, 11.24; S, 8.58. Found: C, 61.00; H, 4.61; N,11.25; S, 8.60.

*N-Allyl-5-(4-fluorophenyl)-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**15**)*

Yield: 19.0%, mp 134–135°C. IR: (cm^{-1}): 3354 (N–H), 1603 (C=N), 1366 (C^4–H), 1328 (C=S), 1096 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.35 (3H; s; Ar–C<u>H</u>$_3$), 3.12 (1H; dd; H_A-pyrazole-H_4), 3.87 (1H; dd; H_B-pyrazole-H_4), 4.16 (2H; m; CH_2), 5.08 (1H; dd; =CH_2, H_a), 5.15 (1H; dd; =CH_2, H_b), 5.88 (1H; m; –CH=, H_x) (J_{ab}: 1.6, J_{ax}: 10.0, J_{bx}: 17.2 Hz), 5.92 (1H; dd; H_X-pyrazole-H_5) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.6 Hz), 7.10–7.80 (8H; m; aromatic prot.), 8.67 (1H; s; CS–N<u>H</u>–CH_2–). ^{13}C NMR (DMSO-d_6); δ 175.96, 161.76 (d, J = 241.1 Hz), 155.29, 141.22, 139.99, 135.88, 129.94, 128.82, 128.07 (d, J = 7.7 Hz), 127.803, 116.14, 115.92 (d, J = 21.2 Hz), 63.27, 46.90, 42.523, 21.75. ESI-MS (*m*/*z*); 376 $[M+Na]^+$ (100%), 354 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{20}FN_3S$: C, 67.96; H, 5.70; N, 11.89; S, 9.07. Found: C, 67.72; H, 5.40; N,11.88; S, 9.26.

*N-Allyl-5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**16**)*

Yield: 20.1%, mp 87–88°C. IR: (cm^{-1}): 3374 (N–H), 1606 (C=N), 1371 (C^4–H), 1334 (C=S), 1139 (C^5–N^1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.08 (1H; dd; H_A-pyrazole-H_4), 3.78 (3H; s; O<u>C</u>H_3), 3.83 (1H; dd; 05 (1H; dd; =CH_2, H_a), 5.12 (1H; dd; =CH_2, H_b), 5.84 (1H; m; –CH=, H_x) (J_{ab}: 1.6, J_{ax}: 10.4, J_{bx}: 17.2 Hz), 5.89 (1H; dd; H_X-pyrazole-H_4) (J_{AB}: 18, J_{AX}: 3.6, J_{BX}: 11.2 Hz), 6.98–7.84 (8H; m; aromatic prot.), 8.59 (1H; s; CS–N<u>H</u>–CH_2–). ESI-MS (*m*/*z*); 392 $[M+Na]^+$ (100%), 370 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{20}FN_3OS$: C, 65.02; H, 5.46; N, 11.37; S, 8.68. Found: C, 64.91; H, 5.31; N,11.63; S, 9.11.

Biochemistry

Chemicals

hMAO-A (recombinant, expressed in baculovirus infected BTI insect cells), hMAO-B (recombinant, expressed in baculovirus infected BTI insect cells), R-(–)-deprenyl hydrochloride, resorufin, dimethyl sulfoxide, and some other chemicals were purchased from Sigma–Aldrich™ (Germany). Moclobemide was a gift (Roche Pharmaceuticals, Germany). The Amplex®-Red MAO Assay Kit (Molecular Probes, USA) contained benzylamine, *p*-tyramine, Clorgyline (MAO-A inhibitor), pargyline (MAO-B inhibitor), and horseradish peroxidase.

Determination of inhibitory activities of the compounds on human MAO-A and -B

The activity of hMAO-A and hMAO-B (using *p*-tyramine as common substrate for both isoforms) was found as 185.60 ± 9.50 pmol/mg/min (n = 3).

The interactions of the synthesized compounds with hMAO isoforms were determined by a fluorimetric method described and modified previously [23–25]. The production of H_2O_2 catalyzed by MAO isoforms was detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex®-Red reagent), a non-fluorescent, highly sensitive, and stable probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce the fluorescent product resorufin.

Kinetic experiments

Newly synthesized compounds were dissolved in dimethyl sulfoxide, maximum concentration 1%, and used in the concentration range of 1–100 μM. Kinetic data for interaction of the enzyme with the compounds were determined using the Microsoft Excel package program. Lineweaver–Burk plots were used to estimate the inhibition constant (K_i) of the inhibitors. SI (K_i (MAO-A)/K_i (MAO-B)) was also calculated. The protein was determined according to the Bradford method [26], in which bovine serum albumin was used as a standard.

Reversibility experiments

Reversibility of the MAO inhibition with novel derivatives was evaluated by a centrifugation-ultrafiltration method [14]. Briefly, adequate amounts of the recombinant hMAO-A or B were incubated together with a single concentration of the newly synthesized compounds or the reference inhibitors in a sodium phosphate buffer (0.05 M, pH 7.4) for 15 min at 37°C. After this incubation period, an aliquot was stored at 4°C and used for the measurement of MAO-A and -B activity. The remaining incubated sample was placed in an Ultrafree-0.5 centrifugal tube (Millipore, USA) with a 30 kDa Biomax membrane in the middle of the tube and centrifuged at 9000 × *g* for 20 min at 4°C. The enzyme retained in the 30 kDa membrane was resuspended in sodium phosphate buffer at 4°C and centrifuged again two successive times. After the third centrifugation, the enzyme retained in the membrane was resuspended in sodium phosphate buffer (300 mL) and an aliquot of this suspension was used for MAO-A and -B activity determination.

Molecular modeling

All calculations were performed by using MOE (The Molecular Operating Environment) Version 2009.10, software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A2R7, http://www.chemcomp.com.

Ligand preparation

The structures of two enantiomers (*R* and *S*) of **4** were constructed using the Builder tool and energy minimized with the MMFF94x forcefield (gradient 0.05).

Docking

The coordinates for the X-ray crystal structure of the enzyme MAO-A and MAO-B were obtained from the RCSB Protein Data Bank (PDB: 2Z5X [22] and 2BK3 [27], respectively). Then properly protonated in the presence of their ligands using the Protonate 3D process in MOE after correcting FAD double bonds. Docking studies for **4** were performed using the Triangle Matcher placement method. Hundred docking iterations were performed for two enantiomers of the ligand and the final refined poses were ranked by the MM/GBVI binding free energy estimation, written in the S field. The best scores of ligand–enzyme complexes were selected and minimized by using the LigX utility which holds the receptor atoms far from the ligand fixed while allowing receptor atoms near the ligand to move.

The authors have declared no conflict of interest.

References

[1] J. Wouters, F. Moureau, G. Evrard, J. J. Koenig, S. Jegham, P. George, F. Durant, *Bioorg. Med. Chem.* **1999**, *7* (*8*), 1683–1693.

[2] O. Curet, G. Damoiseau-Ovens, C. Sauvage, N. Sontag, P. Avenet, H. Depoortere, D. Caille, O. Bergis, B. Scatton, *J. Affect. Disord.* **1998**, *51* (*3*), 287–303.

[3] F. Lotufo-Neto, M. Trivedi, M. E. Thase, *Neuropsychopharmacology* **1999**, *20* (*3*), 226–247.

[4] M. B. Youdim, D. Edmondson, K. F. Tipton, *Nat. Rev. Neurosci.* **2006**, *7* (*4*), 295–309.

[5] M. B. Youdim, Y. S. Bakhle, *Br. J. Pharmacol.* **2006**, *147 (Suppl 1)*, S287–S296.

[6] L. Oreland, *Monoamine Oxidase: Basic Clin. Aspects* **1993**, *1*, 219–247.

[7] M. Ebadi, S. Sharma, S. Shavali, H. El Refaey, *J. Neurosci. Res.* **2002**, *67* (*3*), 285–289.

[8] I. M. Anderson, *Br. Med. Bull.* **2001**, *57* (*1*), 161–178.

[9] J. Knoll, *Neurobiology* **2000**, *8* (*2*), 179–199.

[10] F. Manna, F. Chimenti, A. Bolasco, D. Secci, B. Bizzarri, O. Befani, P. Turini, B. Mondov, S. Alcaro, A. Tafi, *Bioorg. Med. Chem. Lett.* **2002**, *12* (*24*), 3629–3633.

[11] N. Gökhan, A. Yeşilada, G. Ucar, K. Erol, A. A. Bilgin, *Arch. Pharm.* **2003**, *336* (*8*), 362–371.

[12] F. Chimenti, A. Bolasco, F. Manna, D. Secci, P. Chimenti, O. Befani, P. Turini, V. Giovannini, B. Mondovì, R. Cirilli, *J. Med. Chem.* **2004**, *47* (*8*), 2071–2074.

[13] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, O. Befani, P. Turini, S. Alcaro, F. Ortuso, *J. Med. Chem.* **2005**, *48* (*23*), 7113–7122.

[14] F. Chimenti, S. Carradori, D. Secci, A. Bolasco, B. Bizzarri, P. Chimenti, A. Granese, M. Yanez, F. Orallo, *Eur. J. Med. Chem.* **2010**, *45* (*2*), 800–804.

[15] E. Palaska, M. Aytemir, *Eur. J. Med. Chem.* **2001**, *36* (*6*), 539–543.

[16] N. Das, M. Dhanawat, S. K. Shrivastava, *Chem. Pap.* **2012**, *66* (*1*), 67–74.

[17] M. Karuppasamy, M. Mahapatra, S. Yabanoglu, G. Ucar, B. N. Sinha, A. Basu, N. Mishra, A. Sharon, U. Kulandaivelu, V. Jayaprakash, *Bioorg. Med. Chem.* **2010**, *18*, 1875–1881.

[18] E. Palaska, F. Aydin, G. Uçar, D. Erol, *Arch. Pharm.* **2008**, *341* (*4*), 209–215.

[19] W. Davey, D. J. Tivey, *J. Chem. Soc. (Resumed)* **1958**, *1*, 1230–1236.

[20] H. S. Mehra, *J. Indian Chem. Soc.* **1968**, *45*, 178–181.

[21] N. Mishriky, F. Asaad, Y. Ibrahim, A. Girgis, *Pharmazie* **1996**, *51* (*8*), 544–548.

[22] S. Y. Son, J. Ma, Y. Kondou, M. Yoshimura, E. Yamashita, T. Tsukihara, *Proc. Natl. Acad. Sci.* **2008**, *105* (*15*), 5739–5744.

[23] M. Yáñez, N. Fraiz, E. Cano, F. Orallo, *Biochem. Biophys. Res. Commun.* **2006**, *344* (*2*), 688–695.

[24] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, S. Alcaro, F. Ortuso, M. Yáñez, *J. Med. Chem.* **2008**, *51* (*16*), 4874–4880.

[25] M. C. Anderson, F. Hasan, J. M. McCrodden, K. F. Tipton, *Neurochem. Res.* **1993**, *18* (*11*), 1145–1149.

[26] M. M. Bradford, *Anal. Biochem.* **1976**, *72* (*1–2*), 248–254.

[27] F. Hubálek, C. Binda, A. Khalil, M. Li, A. Mattevi, N. Castagnoli, D. E. Edmondson, *J. Biol. Chem.* **2005**, *280* (*16*), 15761–15766.

