Synthesis and Selective Inhibitory Activity of 1-Acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole Derivatives against Monoamine Oxidase

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A novel series of 1-acetyl-3-(4-hydroxy- and 2,4-dihydroxyphenyl)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole derivatives **1**−**12** have been synthesized and investigated for the ability to selectively inhibit the activity of the A and B isoforms of monoamine oxidase (MAO). The new synthesized compounds **1**−**12** proved to be more reversible, potent, and selective inhibitors of MAO-A than of MAO-B. Knowing that stereochemistry may be an important modulator of biological activity, we performed the semipreparative chromatographic enantioseparation of the most potent, selective, and chiral compounds, **6** and **11**. The separated enantiomers were then submitted to in vitro biological evaluation while increasing their inhibitory activity and A selectivity. The (−)-**6** enantiomer shows $K_{i(MAO-A)} = 2$ nM and SI = 165 000, (+)-**6** shows $K_{i(MAO-A)} = 6$ nM and SI = 166 666, (−)-**11** shows $K_{i(MAO-A)} = 4$ nM and SI = 80 000, and (+)-**11** shows $K_{i(MAO-A)} = 7$ nM and SI = 38 571.

Introduction

Amine oxidases (AOs) are widely distributed enzymes among all living organisms with important oxidatively deaminated biological functions, such as biogenic amine metabolism.[1] They are divided into two classes: AOs containing flavin adenine dinucleotide as a cofactor (FAD-AOs), and semicarbazide-sensitive AOs (ssAOs) containing copper(II)−2,4,5-trihydroxyphenylalanine quinone as a cofactor (TPQ-Cu AOs). FAD-AOs, located in the outer membrane of the mitochondria, are named MAOs and exist as two isoforms, MAO-A and MAO-B, which differ in substrate specificity, sensitivity to inhibitors, and amino acid sequence.[2−4] In recent years, there has been considerable renewed interest in MAOs on account of the key role played by the two MAO isoforms in the metabolism of neurotransmitters. As a result, MAO inhibitors (MAOI) are useful in the treatment of several psychiatric and neurological diseases.[5−7] Reversible, selective MAO-A inhibitors are used as antidepressant and antianxiety drugs,[8] and selective MAO-B inhibitors are coadjuvant in the treatment of Parkinson's disease and perhaps also in Alzheimer's disease.[9,10]

Even though the experimental crystal structure of human MAO-B was deposited in the RCSB Protein Data Bank in late 2001,[11] the rational design of potent reversible pharmacophores of MAO inhibitors has not been possible because the three-dimensional structures of the catalytic site of the enzyme are still unknown. A few studies reported in the literature[12−14] have contributed to a better understanding of the factors responsible for MAOI activity and MAO-A/B selectivity. Such studies report that the factors affecting selectivity are fundamentally different. To inhibit the active site of MAO-B, molecule lipophilicity is fundamental, while to inhibit the MAO-A site, a fundamental element is the N5 charge transfer bond of the isoalloxazine nucleus of FAD. Besides these, other equally essential factors are the hydrogen bonds that stabilize the enzyme−inhibitor complex between the polar groups on the inhibitor, the amino acidic residues of the active site of the enzyme, and finally the planar structure of the inhibitor that can best adapt to the receptor pocket.

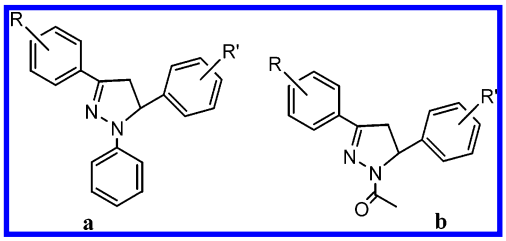

Figure 1. Structures of our previously published pyrazoline derivatives active as AO inhibitors.

It is known that 1,3,5-triphenylpyrazolines show monoamine oxidase inhibitory properties unrelated to tranquillizing, muscle relaxant, psychoanaleptic, or anticonvulsant activities.[15−17] In a previous work we synthesized a series of substituted 1,3,5-triphenyl-4,5-dihydro-(1*H*)-pyrazoles (Figure 1, **a**) which showed reversible and selective inhibitory activity on TPQ-Cu dependent AOs but were inactive on MAOs.[18] In a

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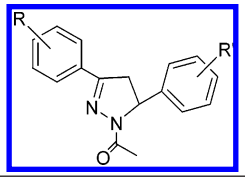
Table 1. Chemicophysical Data of Derivatives **1**−**12**



compound	R	R′	yield %	mp, °C
1	4-OH	2-Cl	82.7	214−5
2	4-OH	3-Cl	57.0	216−8
3	4-OH	4-Cl	73.0	259−6
4	4-OH	3-CH_3	57.2	218−2
5	4-OH	4-CH_3	67.0	235−7
6	4-OH	2-OCH_3	93.5	183−4
7	4-OH	4-OCH_3	90.0	217−8
8	4-OH	2,4-OCH_3	70.0	262−4
9	2,4-OH	4-Cl	75.6	253−5
10	2,4-OH	4-CH_3	70.0	256−8
11	2,4-OH	2-OCH_3	82.5	237−8
12	2,4-OH	4-OCH_3	70.0	253−5

subsequent work by the authors,[19] the substitution of the aromatic ring on N1 of the pyrazoline nucleus with an acetyl group (Figure 1, **b**) favored inhibitory activity toward the MAOs (FAD-dependent AOs). As a matter of fact, this substitution reduces the steric hindrance of the molecules, raising the positive charge of N1 of the heterocycle, which strengthens the charge-transfer bond with the isoalloxazine nucleus of FAD. Though interesting, the inhibitory activity data toward MAOs reported in this work (in the order of 10^{-8}) do not show any selectivity toward the two isoforms. For this reason, we synthesized a new series of 1-acetyl-3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole and 1-acetyl-3-(2,4-dihydroxyphenyl)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole derivatives whose structure contains polar groups capable of forming hydrogen bonds, which as stated earlier, stabilize the bond with FAD while contributing to selectivity toward MAO-A.

Because of the presence of a chiral center at the C5 position of pyrazole moiety, knowing that stereochemistry may be an important modulator of biological activity, we performed the semipreparative chromatographic enantioseparation of the most potent, selective, and active chiral compounds.

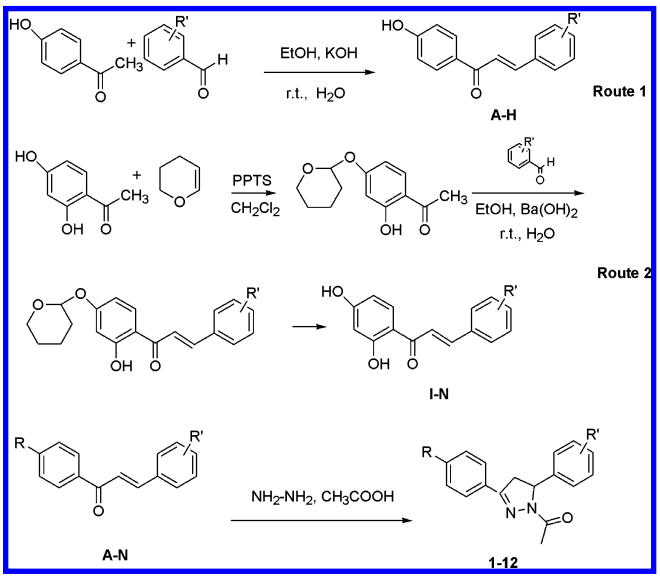
Chemistry

Starting from chalcones **A**−**N**, the new 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives **1**−**12** (Table 1) were obtained by addition of hydrazine hydrate in acetic acid according to a previous method[19] (Scheme 1).

The 4-hydroxy chalcones **A**−**H** were obtained by direct Claisen−Schmidt condensation between the aromatic aldehydes and the substituted acetophenone, using 20% potassium hydroxide as catalyst in ethanol (Route 1 in Scheme 1).

Since the potassium salts precipitated because of the highly alkaline reaction environment due to the synthesis of the 2,4-dihydroxy chalcones **I**−**N**, the hydroxyls had to be protected with 3,4-dihydro-α-pyran before the condensation reaction. Of the two hydroxyls only the one in position 4 is protected, while the one in position 2 is not available, as it is involved in an intramolecular hydrogen bond with the oxygen atom of the carbonyl group. The acetophenone thus protected reacts with the aldehyde to form the protected chalcone, which is subsequently freed by hydrolysis (Route 2 in Scheme 1).

Scheme 1



Chromatographic (HPLC) Resolution of Racemic Samples 6 and 11. The enantiomers of racemic compounds **6** and **11** were separated both on the analytical and the semipreparative scale by enantioselective HPLC on a chiral Chiralcel OD column packed with cellulose carbamate derivative/silica gel.[20] Baseline enantioseparations were achieved within 20 min using *n*-hexane containing 30% vol of ethanol as a mobile phase at a temperature of 25 °C. Flow rates of 1.0 and 3.0 mL min^{-1} for analytical and semipreparative separations, respectively, were used. For the semipreparative runs, 5 mg of racemic sample **6** (dissolved in 500 μL of ethanol) were injected onto a 10 mm I.D. Chiralcel OD chiral stationary phase (CSP). The very poor solubility (<1 mg/mL) of **11** in ethanol and *n*-hexane−alcohol (ethanol or 2-propanol) mixtures precluded injection of more than 0.4 mg (dissolved in 1 mL of ethanol) of racemic **11**. The sign of the optical rotation of the two enantiomers of **6** and **11** was determined by on-line polarimetric detection. The first eluting enantiomers of **6** and **11** were levorotatory at a wavelength of 365 and 589 nm. After semipreparative separations, the collected fractions were analyzed by an analytical Chiralcel OD column (250 × 4.6 mm I.D.) to determine their enantiomeric excess (ee). The first and second eluting enantiomers of **6** had ee = 99.0% and ee = 99.8%, respectively. The enantiomers of **11** were obtained with ee = 99.0% (less retained enantiomer) and ee = 99.8% (more retained enantiomer).

Results and Discussion

All compounds were first evaluated for their ability to inhibit MAO in the presence of kynuramine as a substrate (Table 2). It is interesting to note that all compounds act through the reversible mode, as shown by dialysis performed over 24 h 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. After a first assessment of their inhibitory ability on the MAO, the compounds were tested to determine their activity

Table 2. Monoamine Oxidase Inhibitory Activity of Derivatives **1**−**12**[a]

compound	MAO IC_{50}	MAO-A IC_{50}	MAO-B IC_{50}	SI[b] selectivity
1	$5.0 \times 10^{-5} \pm 0.05$	$8.6 \times 10^{-9} \pm 0.02$	$8.9 \times 0^{-5} \pm 0.03$	10349
2	$1.2 \times 10^{-5} \pm 0.02$	$1.0 \times 10^{-8} \pm 0.01$	$2.5 \times 10^{-5} \pm 0.06$	2500
3	$4.0 \times 10^{-5} \pm 0.09$	$1.0 \times 10^{-7} \pm 0.09$	$4.0 \times 10^{-5} \pm 0.40$	400
4	$9.5 \times 10^{-6} \pm 1.00$	$8.0 \times 10^{-9} \pm 0.05$	$1.9 \times 10^{-5} \pm 0.03$	2375
5	$2.0 \times 10^{-7} \pm 0.05$	$1.0 \times 10^{-7} \pm 0.04$	$3.0 \times 10^{-5} \pm 0.05$	300
6	$3.0 \times 10^{-5} \pm 0.05$	$8.8 \times 10^{-9} \pm 0.01$	$1.0 \times 10^{-4} \pm 0.06$	11363
7	$3.6 \times 10^{-6} \pm 0.03$	$9.0 \times 10^{-9} \pm 0.08$	$7.2 \times 10^{-6} \pm 0.28$	800
8	$1.0 \times 10^{-5} \pm 0.01$	$1.0 \times 10^{-8} \pm 0.25$	$2.0 \times 10^{-5} \pm 0.30$	2000
9	$2.0 \times 10^{-5} \pm 0.06$	$1.0 \times 10^{-8} \pm 0.18$	$1.2 \times 10^{-4} \pm 0.55$	12000
10	$9.5 \times 10^{-6} \pm 0.08$	$9.0 \times 10^{-9} \pm 0.02$	$4.2 \times 10^{-5} \pm 0.07$	4666
11	$4.0 \times 10^{-5} \pm 0.02$	$8.0 \times 10^{-9} \pm 0.01$	$1.3 \times 10^{-4} \pm 0.03$	16250
12	$1.0 \times 10^{-5} \pm 0.04$	$9.0 \times 10^{-9} \pm 0.04$	$8.3 \times 10^{-5} \pm 0.05$	9222

[a] Data represent mean values of at least of three separate experiments. [b] SI: selectivity index = IC_{50} (MAO-B)/IC_{50} (MAO-A).

Table 3. Monoamine Oxidase Inhibitory Activity of Racemate **6**, Enantiomers (−)- and (+)-**6**, Racemate **11**, and Enantiomers (−)- and (+)-**11**[a]

compound	K_i MAO-A	K_i MAO-B	SI[b] selectivity
6	$9.0 \times 10^{-9} \pm 0.010$	$7.6 \times 10^{-4} \pm 0.02$	84444
(−)-**6**	$2.0 \times 10^{-9} \pm 0.015$	$3.3 \times 10^{-4} \pm 0.05$	165000
(+)-**6**	$6.0 \times 10^{-9} \pm 0.020$	$1.0 \times 10^{-3} \pm 0.03$	166666
11	$8.0 \times 10^{-9} \pm 0.050$	$6.0 \times 10^{-4} \pm 0.04$	75000
(−)-**11**	$4.0 \times 10^{-9} \pm 0.016$	$3.2 \times 10^{-4} \pm 0.04$	80000
(+)-**11**	$7.0 \times 10^{-9} \pm 0.020$	$2.7 \times 10^{-4} \pm 0.07$	38571

[a] Data represent mean values of at least of three separate experiments. [b] SI: selectivity index = K_i(MAO-B)/K_i(MAO-A).

toward MAO-A and MAO-B selectively in the presence of the specific substrates, serotonin and benzylamine, respectively (Table 2). This test better shows that some compounds link high inhibitory activity and high A selectivity expressed as IC_{50} (MAO-B)/IC_{50} (MAO-A) ratio in Table 2 (SI = selectivity index).

These biological results indicate not only the influence of the para-substituted hydroxyl group on the aromatic ring bonded to C3 of the pyrazoline ring, but also that of the ortho-substituted methoxyl group on the aromatic ring bonded to C5 of the pyrazoline nucleus.

The data reported in Table 2 show that compounds **1**, **4**, **6**, **7**, **10**, **11**, and **12** all have a high inhibitory activity on MAO-A in the range 8.0×10^{-9} to 9.0×10^{-9}. Interestingly, in particular, compared to others, **6** and **11** are the only compounds that have both the hydroxyl group in para and the methoxyl group in ortho positions. Moreover, these compounds have a high $IC_{50} \geq 10^{-9}$ value that is associated with a high A-selectivity (SI = 11 363 and 16 250, respectively).

Furthermore, because of the presence of a chiral center at the C5 position of the pyrazole moiety, semi-preparative chromatographic enantioseparation of the most potent, selective, and active chiral compounds **6** ($K_{i(MAO-A)}$= 9 nM; SI = 84 444) and **11** ($K_{i(MAO-A)}$= 8 nM; SI = 75 000) was performed. The separated enantiomers were then submitted to in vitro biological evaluation (Table 3). From the results of these experiments it has been possible to point out a difference between the racemic mix and the individual enantiomers in inhibiting the two isoforms selectively. This is particularly evident in compound **6**, for which anti-MAO-A activity varies slightly, while selectivity doubles for the enantiomeric pair, (−)- and (+)-, reaching 165 000 and 166 666, respectively.

In conclusion, we prepared new 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives **1**−**12**, which have been shown to be potent and selective MAO-A inhibitors. Among all compounds, **6** and **11** afford the highest anti-MAO-A activity with maximum A/selectivity especially for the single enantiomer (−)- and (+)- of the compound **6**. The biological results agree with our preliminary hypothesis of a correlation between the presence of polar group/s and high anti-MAO-A activity.

These findings will be taken into account when planning novel, selective MAO-A and MAO-B inhibitors, structurally related to the synthesized compounds **1**−**12**, with the aim of developing a SAR model by a rational design in order to explore in depth the nature of the interactions with the active site.

Experimental Section

Chemistry. Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1605 FT spectrophotometer on potassium bromide mulls. ^{1}H NMR spectra were performed on a Bruker AM-400 (400 MHz) FT spectrometer in $CDCl_3$, and the chemical shifts were reported in ppm referring to the solvent peak. Elemental analyses for C, H, and N were performed on a Carlo Erba model 1106 elemental analyzer, and the analytical results were within ±0.4% of the theoretical values.

General Procedure for the Preparation of 4-Hydroxyphenyl Chalcones A−**H (Route 1).** An aqueous solution of potassium hydroxide (20%, 10 mL) was added under stirring overnight to a solution of the appropriate aryl aldehyde (30 mmol) and 4-hydroxyacetophenone (30 mmol) in 96% ethanol (75 mL) at room temperature. The reaction mixture was then poured into water (100 mL), and after neutralization with hydrochloric acid (5%), a yellow solid was recrystallized from ethanol and identified by observing the formation of the CH=CH at 7.60 and 7.90 ppm in the ^{1}H NMR spectra and the disappearance of the aldehyde proton at 9.87 ppm.

General Procedure for the Preparation of 2,4-Dihydroxyphenyl Chalcones I−**N (Route 2).** A solution of 3,4-dihydro-α-pyran (89.68 mmol) in methylene chloride (50 mL) was added dropwise to a suspension of 2,4-dihydroxyacetophenone (30 mmol) and pyridinium *p*-toluenesulfonate (PPTS) (0.72 mmol) in methylene chloride (150 mL) and stirred at room temperature for 12 h. The reaction mixture was washed with water, and the organic layer dried (Na_2SO_4) and concentrated in vacuo to obtain the protected acetophenone. A solution of 2,4-dihydroxy-4-(tetrahydropyran-2-yloxy)acetophenone (5 mmol) and the appropriate benzaldehyde (5 mmol) in ethanol (12 mL) was slowly added to a solution of barium hydroxide octahydrate (7.43 mmol) in ethanol (60 mL) and stirred for 24 h at 40 °C. The reaction mixture was concentrated in vacuo, washed with water (100 mL), neutralized with 1 M HCl, and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, yielding the crude 2,4-dihydroxy-4-(tetrahydropyran-2-yloxy) chalcone. The compound was suspended in ethanol (30 mL), and *p*-toluenesulfonic acid (0.12 mmol) was added. The reaction mixture was stirred for 12 h at room temperature, diluted with water (30 mL), neutralized with Na_2CO_3, and extracted with ethyl

acetate. The organic layer was dried (Na_2SO_4) and evaporated in vacuo to give the chalcones **I**−**N**.

General Procedure for the Preparation of 1-Acetyl-3-(4-hydroxy- and 2,4-dihydroxyphenyl)-5-phenyl-4,5-dihydro-(1*H*)-pyrazoles 1−12. A solution of chalcones **A**−**N** (5 mmol) in 30 mL of acetic acid was added dropwise to 0.6 mL of hydrazine hydrate (12.5 mmol) and kept under stirring at 120 °C for 24 h. The mixture was then poured in ice−water, obtaining the crude pyrazole derivatives **1**−**12**, which were crystallized from ethanol.

^{1}H NMR and IR Spectral Data of Derivatives 1−12. For all the synthesized compounds, the diagnostic infrared absorptions were 3450−3100 cm^{-1} (OH), 1618 cm^{-1} (C═O), and 1590 cm^{-1} (C═N). As regards ^{1}H NMR spectra, we always detected the following typical peaks for the pyrazoline nucleus: 5.53−5.47 (q, 1H, H_5), 3.89−3.79 (m, 1H, H_4'), and 3.28−3.20 (dd, 1H, H_4). For the 2,4-dihydroxy derivatives **9**−**12**, two different peaks for the hydroxyl groups were observed as broad singlets at 10.26 and 10.04 ppm, which disappeared upon treatment with D_2O. For the 4-hydroxy derivatives **1**−**8**, only a broad singlet at 10.20 ppm, which disappears with D_2O, was observed. For all compounds, **1**−**12**, the aromatic protons appear as multiplets in the range 7.60−6.40 ppm. The methyl group of the *N*1-acetyl was always observed at 2.03 ppm, while for mono- and dimethoxy derivatives **6**−**8** and **12**, a singlet centered at 3.73 ppm was observed. Finally, the methyl-substituted compounds **4**, **5**, and **11** show an additional peak at 2.35 ppm.

Chromatographic (HPLC) Resolution of Racemic Samples 6 and 11. Chiral HPLC of **6** and **11** was performed by stainless steel Chiralcel OD columns (250 × 4.6 mm I.D. and 250 × 10 mm I.D.) (Daicel Chemical Industries, Tokyo, Japan). HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy).

Chiral HPLC was performed using a Waters (Milford, MA) 510 pump equipped with a Rheodyne (Cotati, CA) injector, a 1 mL sample loop, an HPLC Perkin-Elmer (Norwalk, CT) oven, and a Waters Model 996 diode array detector (DAD).

The sign of the optical rotation of the two enantiomers of **6** and **11** was measured on-line at a wavelength of 365 and 589 nm by a Perkin-Elmer polarimeter model 241 equipped with Hg/Na lamps and a 40 μL flow-cell. The system was kept at a constant temperature of 25 °C. The output signal was acquired and processed by Millenium 2010 software.

Biochemistry. All chemicals were commercial reagents of analytical grade and were used without purification. Bovine brain mitochondria were isolated according to Basford.[21] In all experiments the AO activities of the beef brain mitochondria were determined by a fluorimetric method, according to Matsumoto et al.[22] using kinuramine as a substrate at four different final concentrations ranging from 5 μM to 0.1 mM. Briefly, the incubation mixtures contained: 0.1 mL of 0.25 M potassium phosphate buffer (pH 7.4), mitochondria (6 mg/mL), and drug solutions with final concentration ranging from 0 to 10^{-3} μM.

The solutions were incubated at 38 °C for 30 min. Addition of perchloric acid ended the reaction. The samples were centrifuged at 10 000 g for 5 min, and the supernatant was added to 2.7 mL of 0.1 N NaOH. The pyrazole derivatives were dissolved in dimethyl sulfoxide (DMSO) and added to the reaction mixture from 0 to 10^{-3} μM. To study the inhibition of pyrazole derivatives 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 (L-deprenyl 0.5 μM to estimate MAO-A activity, and clorgyline 0.05 μM 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 concentration was determined according to Bradford.[23] The results are reported in Table 2. Dixon plots were used to estimate the inhibition constant (K_i) of the inhibitors; data are reported in Table 3. The data are the means of three or more experiments performed in duplicate.

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References

(1) Mondovì, B. in *Structure and function of amine oxidases*; CRC Press: Boca Raton, FL, 1985.
(2) Johnston, J. P. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **1968**, *17*, 1285−1297.
(3) Weyler, W.; Hsu, Y. P.; Breakefield, O. Biochemistry and genetics of monoamine oxidase. *Pharmacol. Ther.* **1990**, *47*, 391−417.
(4) Geha, R. M.; Rebrin, I.; Chen, K.; Shih, J. C. Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *J. Biol. Chem.* **2001**, *276*, 9877−9882.
(5) Strolin-Benedetti, M.; Dostert, P. L. Monoamine oxidase: From physiology and pathophysiology to the design and clinical application of reversible inhibitors. *Adv. Drug Res.* **1992**, *23*, 65−125.
(6) Youdim, M. B. H.; Finberg, J. P. M. New directions in monoamine oxidase A and B. Selective inhibitors and substrates. *Biochem. Pharmacol.* **1991**, *41*, 155−162.
(7) Cesura, A. M.; Pletscher, A. The new generation of monoamine oxidase inhibitors. *Prog. Drug Res.* **1992**, *38*, 171−297.
(8) Rudorfer, M. V.; Potter, W. Z. Antidepressants. A comparative review of the clinical pharmacology and therapeutic use of the "newer" versus the "older" drugs. *Drugs* **1989**, *37*, 713−738.
(9) Wouters, J. Structural aspects of monoamine oxidase and its reversible inhibition. *Curr. Med. Chem.* **1998**, *5*, 137−162.
(10) Tetrud, J. W.; Langston, J. W. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Science* **1989**, *245*, 519−522.
(11) Binda, C.; Newton-Vinson, P.; Hubálek, F.; Edmondson, D. E.; Mattevi, A. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nat. Struct. Biol.* **2002**, *9*, 22−26.
(12) Wouters, J.; Ooms, F.; Jegham, S.; Koenig, J. J.; George, P.; Durant, F. Reversible inhibition of type B monoamine oxidase. Theoretical study of model diazo heterocyclic compounds. *Eur. J. Med. Chem.* **1997**, *32*, 721−730.
(13) Wouters, J.; Moureau, F.; Evrard, G.; Koenig, J.-J.; Jegham, S.; George, P.; Durant, F. A reversible monoamine oxidase A inhibitor, Befloxatone: structural approach of its mechanism of action. *Bioorg. Med. Chem.* **1999**, *7*, 1683−1693.
(14) Altomare, C.; Cellamare S.; Summo, L.; Catto, M.; Carotti, A. Inhibition of monoamine oxidase-B by condensed pyridazines and pyrimidines: effects of lipophilicity and structure−activity relationships. *J. Med. Chem.* **1998**, *41*, 3812−3820.
(15) Parmar, S. S.; Pandey, B. R.; Dwivedi, C.; Harbison, R. D. Anticonvulsivant activity and monoamine oxidase inhibitory properties of 1,3,5-trisubstituted pyrazolines. *J. Pharm. Sci.* **1974**, *63*, 1152−1155.
(16) Soni, N.; Pande, K.; Kalsi, R.; Kupta, K. T.; Parmar, S. S.; Barthwal, J. P. Inhibition of rat brain monoamine oxidase and succinic dehydrogenase by anticonvulsivant pyrazolines. *Res. Commun. Chem. Pathol. Pharmacol.* **1987**, *56* (1), 129−132.
(17) Palaska, E.; Erol, D.; Demirdamar, R. Synthesis and antidepressant activities of some 1,3,5-triphenyl-2-pyrazolines. *Eur. J. Med. Chem.* **1996**, *31*, 43−47.
(18) Manna, F.; Chimenti, F.; Bolasco, A.; Bizzarri, B.; Befani, O.; Pietrangeli, P.; Mondovì, B.; Turini, P. Inhibitory effect of 1,3,5-triphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives on activity of amine oxidases. *J. Enzyme Inhib.* **1998**, *13*, 207−216.
(19) Manna, F.; Chimenti, F.; Bolasco, A.; Secci, D.; Bizzarri, B.; Befani, O.; Mondovì, B.; Turini, P.; Alcaro, S.; Tafi, A. Inhibition of amine oxidases activity by 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3629−3633.
(20) Yashima, E.; Yamamoto, C.; Okamoto, Y. Polysaccharide-based chiral LC columns. *Synlett.* **1998**, 344−360.
(21) Basford, R. E. Preparation and properties of brain mitochondria. *Methods Enzymol.* **1967**, *10*, 96−101.
(22) Matsumoto, T.; Suzuki, O.; Furuta T.; Asai, M.; Kurokawa, Y.; Rimura, Y.; Katsumata, Y.; Takahashi, I. A sensitive fluorometric assay for serum monoamine oxidase with kynuramine as substrate. *Clin. Biochem.* **1985**, *18*, 126−129.
(23) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

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