

Monoamine Oxidase Inhibitory Properties of Optical Isomers and *N*-substituted Derivatives of 4-methylthioamphetamine

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(±)-4-Methylthioamphetamine (MTA) was resolved into its enantiomers, and a series of *N*-alkyl derivatives of the parent compound, as well as its α -ethyl analogue, were prepared. The monoamine oxidase (MAO) inhibitory properties of these substances were evaluated *in vitro*, using a crude rat brain mitochondrial suspension as the source of enzyme. All compounds produced a selective, reversible and concentration-related inhibition of MAO-A. (+)-MTA proved to be the most potent inhibitor studied, while all the other derivatives were less active than the parent compound, with (–)-MTA being about 18 times less potent than the (+) isomer. The analysis of structure–activity relationships indicates that the introduction of alkyl substituents on the amino group of MTA leads to a reduction in the potency of the derivatives as MAO-A inhibitors, an effect which increases with the size of the substituent.

Keywords: Monoamine oxidase; 4-methylthioamphetamine; MAO inhibitors; Amphetamine derivatives; Structure–activity relationships; Antidepressants

INTRODUCTION

Monoamine oxidase (EC 1.4.3.4, amine: O₂ oxidoreductase, MAO), which catalyzes the oxidative deamination of a variety of monoamines such as catecholamines and serotonin (5-HT), exists in two different isoforms (MAO-A and -B) that are distinguishable by their substrate selectivity,

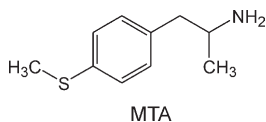
inhibitor sensitivity and amino acid sequence.^{1–3} Non-selective and irreversible MAO inhibitors (MAOI) have been used as effective antidepressants, but severe side effects have limited their use.^{4–6} More recently developed, selective and reversible inhibitors of the A-isoform (MAOI-A) exhibit antidepressant actions while they appear to have less side effects than their irreversible predecessors.^{7–9}

An extensive series of phenylisopropylamines, including amphetamine itself, has been evaluated as MAOI.^{10–14} Several of these studies have assessed the influence of different substituents on the aromatic ring of the phenylisopropylamine molecule (in particular at the *para* position), upon MAO inhibitory properties. However, there is no consistent information about how the alkylation of the amino group of phenylisopropylamines might modify the potency and selectivity of these molecules.

Several years ago, (±)-4-methylthioamphetamine (MTA) was shown to be a potent, selective, non-neurotoxic inducer of serotonin (5-HT) release from rat frontal cortical slices.¹⁵ More recently, microdialysis studies in anesthetized rats showed that MTA produces a rapid and persistent increase in 5-HT basal release in the hippocampus, and this drug was also found to be a potent, selective, reversible inhibitor of MAO-A,^{14,16} suggesting that it might serve as a lead for the development of novel antidepressants with a short onset of action. All these results, however, were obtained with the racemic

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modification of the drug. In addition, MTA has recently gained certain notoriety as a street drug, and one case of poisoning has been attributed to its ingestion.^{17,18,19}



Based on these precedents, the goal of the present study was to evaluate the MAO inhibitory properties of the optical isomers and of a series of *N*-alkyl derivatives of MTA, as well as its α -ethyl homologue, in order to contribute to a better understanding of the structural requirements necessary for effective inhibition of the enzyme by phenylisopropylamines. Finally, some structure–activity relationships for this series of drugs are discussed.

MATERIALS AND METHODS

Reagents

All the chemicals used were of the highest grade commercially available. Tetrahydrofuran and acetonitrile were Merck HPLC grade. 5-HT, 5-hydroxyindoleacetic acid, yeast aldehyde dehydrogenase and β -nicotinamide dinucleotide were from Sigma (St. Louis, MO, USA). *l*-Deprenyl was generously donated by Prof. J. Knoll (Semmelweis University of Medicine, Hungary). 4-Methylthiobenzaldehyde, 4-dimethylaminophenylacetic acid and all other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

General Methods

Melting points were determined on a Reichert-Jung Galen III Kofler hot stage and are uncorrected. Optical rotations were determined with a Schmidt-Haensch Polartronic electronic polarimeter. NMR spectra were recorded in CDCl₃ or D₂O using a Bruker AMX 300 instrument, operating at 300.13 (¹H) or 75.03 (¹³C) MHz. Chemical shifts are reported in δ values (ppm relative to tetramethylsilane as an internal reference).

Synthesis

(±)-1-(4-Methylthiophenyl)-2-aminopropane (MTA)

A mixture of 4-methylthiobenzaldehyde (10 mL, 75 mmol), dry ammonium acetate (6.0 g, 81 mmol), nitroethane (55 mL) and toluene (100 mL) was refluxed overnight under a Dean–Stark trap. The mixture was concentrated to 70 mL and stored overnight at –20°C. 1-(4-Methylthiophenyl)-2-nitropropene

was collected by filtration (8.5 g) as bright yellow crystals. The mother liquors were evaporated under reduced pressure, and the dark reddish brown oily residue was diluted with hot methanol (80 mL). On cooling, another fraction of the nitropropene crystallized (5.3 g) sufficiently pure for use in the next synthetic step. After removal of the solvent, the residue was partitioned between H₂O and CHCl₃, and the organic layer was dried with Na₂SO₄, concentrated and fractionated over a silica gel column, eluting with CHCl₃. In this way, additional nitropropene was recovered (0.5 g) which was recrystallized from methanol (total yield 91%) m.p. 73–74.5°C; ¹H NMR (CDCl₃) δ 2.46 (s, 3H, S-CH₃), 2.52 (s, 3H, C-CH₃), 7.28–7.36 (A₂B₂ system, 4H, *J* = 8 Hz, Ar-*H*), 8.04 (s, 1H, Ar-CH); ¹³C NMR (CDCl₃) δ 14.09 (S-CH₃), 14.95 (C-1), 125.81 (C-2'/C-6'), 128.51 (C-2), 130.44 (C-3'/C-5'), 142.12 (C-1'), 146.80 (C-4'). A solution of the nitropropene (13.0 g, 62 mmol) in dry THF (100 mL) was added dropwise to a stirred suspension of LiAlH₄ (13.0 g, 342 mmol) in dry THF (200 mL). The reaction mixture was refluxed in an oil bath for 24 h and then cooled in an ice/water bath and the reaction quenched by cautious addition of H₂O (15 mL), 40% NaOH (13 mL) and again H₂O (45 mL). The suspension was filtered, rinsing the cake well with THF. The solvent was removed under reduced pressure to yield MTA as a light yellow oil which was distilled under vacuum (150°C, 0.1 Torr) giving a colorless product (10.0 g, 55 mmol, 81%). ¹H NMR (CDCl₃) δ 1.08 (d, 3H, *J* = 6.3 Hz, CHCH₃), 2.44 (s, 3H, S-CH₃), 2.47 (dd, 1H, *J* = 13.4 Hz, *J'* = 8.0, CH₂CH), 2.64 (dd, 1H, *J* = 12.8, *J'* = 7.9 Hz, CH₂CH), 3.11 (m, 1H, 6.3 Hz, CH₃CH), 7.08–7.19 (A₂B₂ system, 4H, *J* = 8.2 Hz, Ar-*H*); MTA·HCl (*i*-PrOH-Et₂O) m.p. 190–192°C, ¹H NMR (D₂O) δ 1.22 (d, 3H, *J* = 6.6 Hz, CHCH₃), 2.42 (s, 3H, S-CH₃), 2.84 (d, 2H, *J* = 7.1 Hz, CH₂CH), 3.55 (m, 1H, 6.8 Hz, CH₃CH), 7.19 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.36 (d, 2H, *J* = 8.2 Hz, Ar-*H*). Found C, 55.20; H, 7.42; N, 6.68; S, 12.84. C₁₀H₁₆ClNS requires C, 55.16; H, 7.41; N, 6.43; S, 14.72%.

(±)-*N*-Methyl-1-(4-methylthiophenyl)-2-aminopropane (NMMTA)

To a solution of MTA (1.8 g, 9.7 mmol) in CHCl₃ (30 mL), ethyl chloroformate (5 mL, 52 mmol) was added. The reaction was refluxed for 8 h and the solvent was removed to give *N*-ethoxycarbonyl-MTA (2.2 g, 89%) as a colorless oil which was used without further purification; ¹H NMR (CDCl₃) δ 1.10 (d, 3H, *J* = 6.6 Hz, CHCH₃), 1.21 (t, 3H, *J* = 7.1 Hz, O-CH₂CH₃), 2.48 (s, 3H, S-CH₃), 2.64 (dd, 1H, *J* = 13.5 Hz, *J'* = 7.1, CH₂CH), 2.80 (dd, 1H, *J* = 13.5, *J'* = 5.6 Hz, CH₂CH), 3.93 (m, 1H, CH₃CH), 4.08 (q, 2H, *J* = 7.1 Hz, O-CH₂CH₃), 4.52 (br s, 1H, N-H), 7.10 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-*H*). To a stirred suspension of LiAlH₄ (2.6 g, 68 mmol) in

anhydrous ethyl ether (150 mL), a solution of the former product (3.0 g, 12 mmol) in anhydrous ethyl ether (50 mL) was added. The mixture was refluxed for 24 h and the excess hydride was destroyed by addition of dilute H₂SO₄ (20 g in 500 mL H₂O) until a clear solution was obtained. The organic phase was separated and the aqueous layer was extracted with ethyl ether (70 mL) and then with CH₂Cl₂ (2 × 80 mL). The mixture was made basic to pH 9 with 40% NaOH and extracted with CH₂Cl₂ (3 × 150 mL). These extracts were pooled, dried with anhydrous Na₂SO₄ and the solvent removed under vacuum. The residual light yellow oil was distilled under reduced pressure (150°C, 0.1 Torr) to afford a colorless product (1.5 g, 68%). ¹H NMR (CDCl₃) δ 1.04 (d, 3H, J = 6.1 Hz, CHCH₃), 2.43 (s, 3H, N-CH₃), 2.47 (s, 3H, S-CH₃), 2.58 (dd, 1H, J = 12.9 Hz, J' = 6.3 Hz, CH₂CH), 2.68 (dd, 1H, J = 13.2 Hz, J' = 6.8 Hz, CH₂CH), 2.75 (m, 1H, J = 6.4 Hz, CH₃CH), 7.11 (d, 2H, J = 8.2 Hz, Ar-H), 7.20 (d, 2H, J = 8.2 Hz, Ar-H). NMMTA·HCl (*i*-PrOH-Et₂O) m.p. 169–171°C; ¹H NMR (D₂O) δ 1.22 (d, 3H, J = 6.6 Hz, CHCH₃), 2.46 (s, 3H, S-CH₃), 2.67 (s, 3H, N-CH₃), 2.82 (dd, 1H, J = 13.8 Hz, J' = 6.3 Hz, CH₂CH), 3.01 (dd, 1H, J = 13.8 Hz, J' = 6.1 Hz, CH₂CH), 3.47 (m, 1H, J = 6.4 Hz, CH₃CH), 7.22 (d, 2H, J = 8.2 Hz, Ar-H), 7.30 (d, 2H, J = 8.2 Hz, Ar-H). Found C, 57.09; H, 7.88; N, 6.31; S, 12.40. C₁₁H₁₈ClNS requires C, 57.00; H, 7.83; N, 6.05; S, 13.83%.

(±)-N,N-Dimethyl-1-(4-methylthiophenyl)-2-aminopropane (DMMTA)

To a stirred solution of MTA hydrochloride (0.20 g, 0.11 mmol) in a mixture of MeOH (8 mL) and 40% formaldehyde solution (2.4 mL) was added NaCNBH₄ (0.90 g, 14 mmol). The pH was kept near 6 by the occasional addition of HCl. When the pH was stable (after about 48 h), the reaction mixture was poured into H₂O (70 mL) and made strongly basic by the addition of aqueous NaOH. The solution was extracted with CH₂Cl₂ (3 × 20 mL), the extracts pooled, and extracted with dilute H₂SO₄ (3 × 20 mL). The pooled acidic extracts were made basic and again extracted with CH₂Cl₂, and the solvent was removed under vacuum to give 0.17 g (73%) of a colorless oil. ¹H NMR (CDCl₃) δ 1.00 (d, 3H, J = 6.6 Hz, CHCH₃), 2.43 (dd, 1H, J = 12.9 Hz, J' = 2.8 Hz, CH₂CH), 2.45 (s, 6H, N-(CH₃)₂), 2.47 (s, 3H, S-CH₃), 2.95 (m, 1H, CH₃CH), 3.10 (dd, 1H, J = 13.0 Hz, J' = 4.2 Hz, CH₂CH), 7.11 (d, 2H, J = 8.2 Hz, Ar-H), 7.20 (d, 2H, J = 8.2 Hz, Ar-H). DMMTA·HCl (*i*-PrOH-Et₂O) m.p. 163–165°C; ¹H NMR (D₂O) δ 1.18 (d, 3H, J = 6.6 Hz, CHCH₃), 2.44 (s, 3H, S-CH₃), 2.80 (s, 6H, N-(CH₃)₂, partially masking m, 1H, CH₂CH), 3.06 (dd, 1H, J = 13.6 Hz, J' = 5.2 Hz, CH₂CH), 3.61 (m, 1H, CH₃CH), 7.22 (d, 2H, J = 8.2 Hz, Ar-H), 7.28 (d, 2H, J = 8.2 Hz, Ar-H). Found C, 58.53; H, 8.21; N,

5.95; S, 10.88. C₁₂H₂₀ClNS requires C, 58.64; H, 8.20; N, 5.70; S, 13.04%.

(±)-N-Ethyl-1-(4-methylthiophenyl)-2-aminopropane (NEMTA)

To a stirred solution of MTA (1.0 g, 5.5 mmol) in pyridine (5.2 mL), was added acetic anhydride (0.7 g, 6.7 mmol) and the mixture was stirred at room temperature for 30 min. The mixture was poured into H₂O (60 mL) and made acidic with HCl. The aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL), the extracts pooled, washed with dilute HCl and dried with anhydrous Na₂SO₄. The solvent was removed under vacuum to give 1.1 g (89%) of 1-(4-methylthiophenyl)-2-acetamidopropane, pure enough for the next synthetic step. ¹H NMR (CDCl₃) δ 1.09 (d, 3H, J = 6.7 Hz, CHCH₃), 1.93 (s, 3H, COCH₃), 2.47 (s, 3H, S-CH₃), 2.67 (dd, 1H, J = 13.5 Hz, J' = 7.2 Hz, CH₂CH), 2.79 (dd, 1H, J = 13.6 Hz, J' = 5.7 Hz, CH₂CH), 4.23 (m, 1H, J = 6.9 Hz, CH₃CH), 7.09 (d, 2H, J = 8.2 Hz, Ar-H), 7.20 (d, 2H, J = 8.2 Hz, Ar-H). To a stirred suspension of LiAlH₄ (1.0 g, 26 mmol) in anhydrous THF (80 mL), a solution of the former product (1.1 g, 4.9 mmol) in anhydrous THF (5 mL) was added. The mixture was refluxed for 4 days and the excess hydride was carefully destroyed by addition of H₂O (1 mL), followed by 40% NaOH (1 mL) and additional H₂O (3 mL). The solid was filtered and washed with THF (20 mL). The solvent was removed under vacuum to give 0.92 g of a light brown oil. This product was distilled under vacuum (180°C, 0.1 mm Hg) yielding 0.75 g (73%) of a colorless oil. ¹H NMR (CDCl₃) δ 1.06 (overlapping d and t, 6H, CHCH₃ and N-CH₂CH₃), 2.46 (s, 3H, S-CH₃), 2.58 (overlapping m, 2H, N-CH₂CH₃ and CH₂CH), 2.71 (overlapping m, 2H, N-CH₂CH₃ and CH₂CH), 2.89 (m, 1H, J = 6.4 Hz, CH₃CH), 7.10 (d, 2H, J = 8.2 Hz, Ar-H), 7.20 (d, 2H, J = 8.2 Hz, Ar-H). NEMTA·HCl (*i*-PrOH-Et₂O) m.p. 184–187°C; ¹H NMR (D₂O) δ 1.2 (overlapping d and t, 6H, CHCH₃ and N-CH₂CH₃), 2.44 (s, 3H, S-CH₃), 2.74 (dd, 1H, J = 13.5 Hz, J' = 8.9 Hz, CH₂CH), 3.08 (overlapping m, 3H, N-CH₂CH₃ and CH₂CH), 3.48 (m, 1H, J = 6.4 Hz, CH₃CH), 7.20 (d, 2H, J = 8.2 Hz, Ar-H), 7.28 (d, 2H, J = 8.2 Hz, Ar-H). Found C, 58.56; H, 8.23; N, 5.97; S, 11.42. C₁₂H₂₀ClNS requires C, 58.64; H, 8.20; N, 5.70; S, 13.04%.

(±)-N,N-Diethyl-1-(4-methylthiophenyl)-2-aminopropane (DEMTA)

To molecular sieves (1.0 g) in DMF (15 mL), KOH (0.25 g, 4.5 mmol) was added and the suspension was vigorously stirred for 10 min. MTA (0.8 g, 4.5 mmol) was added and stirring was continued for 30 min. Finally, iodoethane (1.0 mL, 7.7 mmol) was added. The reaction mixture was left at room temperature

for 24 h. The solid was filtered and washed with EtOAc. The solvent was removed under vacuum, NaOH (2N, 40 mL) was added and the mixture was extracted with EtOAc (4 × 20 mL). The extracts were pooled, washed with saturated brine and dried with anhydrous Na₂SO₄. The solvent was removed under vacuum and the crude mixture was purified over a flash column eluting with CHCl₃/MeOH/NH₃ (90/9/1), to give 0.37 g (35%) of DEMTA. ¹H NMR (CDCl₃) δ 0.91 (d, 3H, *J* = 6.5 Hz, CHCH₃), 1.05 (t, 6H, *J* = 7.1 Hz, N-(CH₂CH₃)₂), 2.35 (dd, 1H, *J* = 12.9 Hz, *J'* = 9.4 Hz, CH₂CH), 2.46 (s, 3H, S-CH₃), 2.54 (m, 4H, N-(CH₂CH₃)₂), 2.88 (dd, 1H, *J* = 12.7 Hz, *J'* = 4.2 Hz, CH₂CH), 2.98 (m, 1H, *J* = 6.5 Hz, CH₃CH), 7.09 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.19 (d, 2H, *J* = 8.2 Hz, Ar-*H*). DEMTA·HBr (*i*-PrOH-Et₂O) m.p. 149–151°C; ¹H NMR (D₂O) δ 1.19 (d, 3H, *J* = 6.7 Hz, CHCH₃), 1.29 (t, 6H, *J* = 5.7 Hz, N-(CH₂CH₃)₂), 2.45 (s, 3H, S-CH₃), 2.81 (dd, 1H, *J* = 13.4 Hz, *J'* = 9.8 Hz, CH₂CH), 3.08 (dd, 1H, *J* = 13.5 Hz, *J'* = 5.0 Hz, CH₂CH), 3.24 (m, 4H, N-(CH₂CH₃)₂), 3.72 (m, 1H, *J* = 6.3 Hz, CH₃CH), 7.24 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.32 (d, 2H, *J* = 8.2 Hz, Ar-*H*). Found C, 52.92; H, 7.68; N, 4.63; S, 8.36; C₁₄H₂₄BrNS requires C, 52.83; H, 7.60; N, 4.40; S, 10.07%.

N-Propyl-1-(4-methylthiophenyl)-2-aminopropane (NPMTA)

To molecular sieves (1.0 g) in DMF (15 mL), KOH (0.25 g, 4.5 mmol) was added and the suspension was vigorously stirred for 10 min. MTA (0.8 g, 4.5 mmol) was added and stirring was continued for 30 min. Finally, bromopropane (1.0 mL, 7.7 mmol) was added. The reaction mixture was left at room temperature for 24 h. The solid was filtered and washed with EtOAc. The solvent was removed under vacuum, NaOH (2N, 40 mL) was added and the mixture was extracted with EtOAc (4 × 20 mL). The extracts were pooled, washed with saturated brine and dried with anhydrous Na₂SO₄. The solvent was removed under vacuum and the crude mixture was purified over a flash column eluting with CHCl₃/MeOH/NH₃ (94/5/1), to give 0.37 g (35%) of NPMTA. ¹H NMR (CDCl₃) δ 0.87 (t, 3H, *J* = 7.4 Hz, N-CH₂CH₂CH₃), 1.05 (d, 3H, *J* = 6.2 Hz, CHCH₃), 1.45 (m, 2H, N-CH₂CH₂CH₃), 2.46 (s, 3H, S-CH₃), 2.5–2.7 (overlapping m, 4H, N-CH₂CH₂CH₃ and CH₂CH), 2.87 (m, 1H, CHCH₃), 7.10 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-*H*). NPMTA·HCl (*i*-PrOH-Et₂O) m.p. 173–175°C; ¹H NMR (D₂O) δ 0.91 (t, 3H, *J* = 7.5 Hz, N-CH₂CH₂CH₃), 1.20 (d, 3H, *J* = 6.6 Hz, CHCH₃), 1.63 (m, 2H, N-CH₂CH₂CH₃), 2.45 (s, 3H, S-CH₃), 2.76 (dd, 1H, *J* = 13.7 Hz, *J'* = 8.9 Hz, CH₂CH), 3.0 (overlapping m, 3H, N-CH₂CH₂CH₃ and CH₂CH), 3.50 (m, 1H, CH₃CH), 7.22 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.30 (d, 2H, *J* = 8.3 Hz, Ar-*H*). Found C, 59.82; H, 8.35; N, 5.79; S,

10.93. C₁₃H₂₂ClNS requires C, 60.32; H, 8.18; N, 5.41; S, 12.38%.

N-Allyl-1-(4-methylthiophenyl)-2-aminopropane (NAMTA) And *N,N*-diallyl-1-(4-methylthiophenyl)-2-aminopropane (DAMTA)

To molecular sieves (0.3 g) in DMF (6 mL) was added KOH (0.06 g, 1.1 mmol) and the suspension was vigorously stirred for 10 min. MTA (0.20 g, 1.1 mmol) was added and stirring was continued for 30 min. Finally, allyl bromide (0.3 mL, 3.3 mmol) was added. The reaction mixture was left at room temperature for 24 h. The solid was filtered and washed with EtOAc. The solvent was removed under vacuum, NaOH (2N, 40 mL) was added, and the mixture was extracted with EtOAc (4 × 20 mL). The extracts were pooled, washed with saturated brine and dried with anhydrous Na₂SO₄. The solvent was removed under vacuum and the mixture of products was fractionated over a flash column eluting with (EtOAc/EtOH 9:1) to give 0.06 g (25%) of NAMTA and 0.14 g (49%) of DAMTA. NAMTA: ¹H NMR (CDCl₃) δ 1.06 (d, 3H, *J* = 6.3 Hz, CHCH₃), 2.47 (s, 3H, S-CH₃), 2.59 (dd, 1H, *J* = 13.4 Hz, *J'* = 6.9 Hz, CH₂CH), 2.76 (dd, 1H, *J* = 13.4 Hz, *J'* = 6.5 Hz, CH₂CH), 2.95 (m, 1H, *J* = 6.5 Hz, CH₃CH), 3.23 (dd, 1H, *J* = 13.9 Hz, *J'* = 5.4 Hz, N-CH₂CH=CH₂), 3.34 (dd, 1H, *J* = 13.9 Hz, *J'* = 5.9 Hz, N-CH₂CH=CH₂), 5.09 (dd, 1H, *J* = 13.8 Hz, *J'* = 1.2 Hz, *cis*CH=CH₂), 5.15 (dd, 1H, *J* = 17.2 Hz, *J'* = 1.5 Hz, *trans*CH=CH₂), 5.86 (m, 1H, CH=CH₂), 7.11 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-*H*). NAMTA·HCl (*i*-PrOH-Et₂O) m.p. 171–173°C; ¹H NMR (D₂O) δ 1.22 (d, 3H, *J* = 6.6 Hz, CHCH₃), 2.46 (s, 3H, S-CH₃), 2.78 (dd, 1H, *J* = 13.8 Hz, *J'* = 8.7 Hz, CH₂CH), 3.07 (dd, 1H, *J* = 13.8 Hz, *J'* = 5.6 Hz, CH₂CH), 3.54 (dd, 1H, *J* = 14.0 Hz, *J'* = 6.3 Hz, N-CH₂CH=CH₂), 3.66 (overlapping m, 2H, CH₃CH and N-CH₂CH=CH₂), 5.43 (d, 1H, *J* = 3.2 Hz, *cis*CH=CH₂), 5.48 (d, 1H, *J* = 10.1 Hz, *trans*CH=CH₂), 5.85 (m, 1H, CH=CH₂), 7.22 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.30 (d, 2H, *J* = 8.2 Hz, Ar-*H*). Found C, 60.62; H, 7.84; N, 5.74; S, 10.83. C₁₃H₂₀ClNS requires C, 60.56; H, 7.82; N, 5.43; S, 12.44%.

DAMTA: ¹H NMR (CDCl₃) δ 0.92 (d, 3H, *J* = 6.6 Hz, CHCH₃), 2.36 (dd, 1H, *J* = 13.1 Hz, *J'* = 9.0 Hz, CH₂CH), 2.46 (s, 3H, S-CH₃), 2.87 (dd, 1H, *J* = 13.1 Hz, *J'* = 5.1 Hz, CH₂CH), 3.09 (overlapping m, 5H, CH₃CH and N-(CH₂CH=CH₂)₂), 5.08 (apparent d, 2H, *J* = 10.0 Hz, *cis*CH=CH₂), 5.16 (dd, 2H, *J* = 17.2 Hz, *J'* = 5.2 Hz, *trans*CH=CH₂), 5.84 (m, 2H, CH=CH₂), 7.06 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.17 (d, 2H, *J* = 8.2 Hz, Ar-*H*). DAMTA·HCl (*i*-PrOH-Et₂O) m.p. 136–138°C; ¹H NMR (D₂O) δ 1.25 (d, 3H, *J* = 6.6 Hz, CHCH₃), 2.46 (s, 3H, S-CH₃), 2.81 (dd, 1H, *J* = 13.3 Hz, *J'* = 10.0 Hz, CH₂CH), 3.11 (dd, 1H, *J* = 13.5 Hz, *J'* = 4.7 Hz, CH₂CH), 3.76

(overlapping m, 5H, CH₃CH and N-(CH₂-CH=CH₂)₂), 5.58 (m, 2H, *cis*CH=CH₂), 5.58 (m, 2H, *trans*CH=CH₂), 5.58 (m, 2H, CH=CH₂), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.28 (d, 2H, *J* = 8.2 Hz, Ar-H). Found C, 64.29; H, 7.99; N, 5.06; S, 8.91. C₁₆H₂₅ClNS requires C, 64.51; H, 8.12; N, 4.70; S, 10.76%.

N,N-Dipropyl-1-(4-methylthiophenyl)-2-aminopropane (DPMTA)

DAMTA (1.2 g, 4.59 mmol) was hydrogenated catalytically with Pd-C 10% in methanol for 24 h, after that the catalyst was removed by filtration over celite. The solvent was removed under vacuum to give 1.1 g (90 %). ¹H NMR (CDCl₃) δ 0.85 (t, 6H, *J* = 7.4 Hz, N-(CH₂CH₂CH₃)₂), 0.90 (d, 3H, *J* = 6.5 Hz, CHCH₃), 1.42 (sex, 4H, *J* = 7.4 Hz, N-(CH₂-CH₂CH₃)₂), 2.3–2.4 (overlapping m, 5H, N-(CH₂-CH₂CH₃)₂ and CH₂CH), 2.44 (s, 3H, S-CH₃), 2.8–3.0 (overlapping m, 2H, CH₂CH and CHCH₃), 7.08 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.17 (d, 2H, *J* = 8.2 Hz, Ar-H). DPMTA·Malonate (*i*-PrOH-Et₂O) m.p. 109–111°C; ¹H NMR (D₂O) δ 0.91 (t, 3H, *J* = 7.3 Hz, N-CH₂CH₂CH₃), 0.94 (t, 3H, *J* = 7.3 Hz, N-CH₂CH₂CH₃), 1.20 (d, 3H, *J* = 6.6 Hz, CHCH₃), 1.7 (m, 4H, N-(CH₂CH₂CH₃)₂), 2.45 (s, 3H, S-CH₃), 2.82 (dd, 1H, *J* = 13.4, *J'* = 9.7 Hz, CH₂CH), 3.0–3.3 (overlapping m, 5H, N-(CH₂CH₂CH₃)₂ and CH₂CH), 3.72 (m, 1H, CHCH₃), 7.23 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.30 (d, 2H, *J* = 8.3 Hz, Ar-H); ¹H NMR (DMSO-*d*₆) δ 0.91 (t, 6H, *J* = 7.4 Hz, N-CH₂CH₂CH₃), 1.07 (d, 3H, *J* = 6.4 Hz, CHCH₃), 1.62 (m, 4H, N-(CH₂CH₂CH₃)₂), 2.46 (s, 3H, S-CH₃), 2.63 (dd, 1H, *J* = 13.8 Hz, *J'* = 6.4 Hz, CH₂CH), 2.85 (s, 2H, CH₂(CO₂H)₂), 2.95 (dd, 1H, *J* = 13.8, *J'* = 9.5 Hz, CH₂CH), 2.80–2.94 (m, 4H, N-(CH₂CH₂CH₃)₂), 3.87 (m, 1H, CHCH₃), 7.21 (s, 4H, Ar-H). Found C, 60.96; H, 8.30; N, 3.96; S, 7.82. C₁₉H₃₁NO₄S requires C, 61.76; H, 8.46; N, 3.79; S, 8.68%.

1-(4-Methylthiophenyl)-2-aminobutane (MTAB)

A mixture of 4-methylthiobenzaldehyde (1.3 mL, 10 mmol), *N,N*-dimethylethylenediamine (1.1 mL, 10 mmol), nitropropane (4.5 mL, 41 mmol) and toluene (10 mL) was refluxed overnight under a Dean-Stark trap. The solvent was removed under vacuum and the crude mixture (1.2 g, 54%) was fractionated over a flash column, eluting with CHCl₃, to obtain (*E*)-1-(4-methylthiophenyl)-2-nitrobutene (1.1 g, 91% of the mixture) and its (*Z*) isomer (0.1 g, 9% of the mixture), each geometrical isomer identified by its NOESY spectrum. (*E*) isomer ¹H NMR (CDCl₃) δ 1.28 (t, 3H, *J* = 7.4 Hz, CH₂CH₃), 2.52 (s, 3H, S-CH₃), 2.88 (q, 2H, *J* = 7.4 Hz, CH₃CH₂C=CH), 7.29 (d, 2H, *J* = 8.5 Hz, H3' and H5'), 7.36 (d, 2H, *J* = 8.5 Hz, H2' and H6'), 7.98 (s, 1H, Ar-CH=C). (*Z*) isomer ¹H NMR (CDCl₃) δ 1.20 (t, 3H, *J* = 7.4 Hz, CH₂CH₃), 2.46

(s, 3H, S-CH₃), 2.67 (q, 2H, *J* = 7.4 Hz, CH₃CH₂-C=CH), 6.29 (s, 1H, Ar-CH=C), 7.16 (s, 4H, *J* = 9.2 Hz, Ar-H). To a stirred suspension of LiAlH₄ (2.2 g, 58 mmol) in anhydrous THF (80 mL), a solution of the (*Z*) and (*E*) nitrobutene mixture (2.3 g, 10 mmol) in anhydrous THF (20 mL) was added dropwise. The mixture was refluxed for 24 h. The excess hydride was carefully destroyed by cautious addition of H₂O (2.5 mL), 40% NaOH (2 mL) and additional H₂O (8 mL). The solid was filtered and washed with THF (20 mL). The solvent was removed under vacuum to obtain MTAB as a light brown oil, which was distilled under vacuum (155–160°C, 0.1 mmHg) yielding a colorless oil (1.8 g, 9.3 mmol, 90%). ¹H NMR (CDCl₃) δ 0.97 (t, 3H, *J* = 7.4 Hz, CH₂CH₃), 1.44 (m, 2H, CH₂CH₃), 2.41 (dd, 1H, *J* = 13.4 Hz, *J'* = 8.6 Hz, CH₂CH), 2.43 (s, 3H, S-CH₃), 2.75 (dd, 1H, *J* = 13.4 Hz, *J'* = 4.7 Hz, CH₂CH), 2.87 (m, 1H, CH₃CH₂-CH), 7.11 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-H). MTAB·HCl (*i*-PrOH-Et₂O) m.p. 159–161°C; ¹H NMR (D₂O) δ 0.95 (t, 3H, *J* = 7.4 Hz, CH₂CH₃), 1.65 (qd, 2H, *J* = 14.7 Hz, *J'* = 7.6 Hz, CH₂CH₃), 2.43 (s, 3H, S-CH₃), 2.78 (dd, 1H, *J* = 14.3 Hz, *J'* = 7.9 Hz, CH₂CH), 2.96 (dd, 1H, *J* = 14.3 Hz, *J'* = 6.2 Hz, CH₂CH), 3.40 (m, 1H, *J* = 6.5 Hz, CH₃CH₂CH), 7.20 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.27 (d, 2H, *J* = 8.2 Hz, Ar-H). Found C, 56.85; H, 7.82; N, 6.29; S, 12.09. C₁₁H₁₈ClNS requires C, 57.00; H, 7.83; N, 6.05; S, 13.83%.

Enantiomeric Resolution of MTA

A solution of (±)-MTA (3.1 g, 17 mmol) in *i*-PrOH (10 mL) was added to a solution of (+)-*O,O'*-di-*p*-toluoyltartaric acid (6.7 g, 17 mmol) in boiling *i*-PrOH (15 mL), and the reaction mixture was boiled for 5 min. After cooling, the precipitated salt was filtered and dried for 48 h in a vacuum over H₂SO₄, and then for 2 h at 66°C. The mixture of diastereomeric MTA salts (7.2 g, 73%) was then dissolved in boiling MeOH (620 mL). This solution was allowed to cool to room temperature and then kept at –20°C for a fortnight. The precipitated salt was filtered and dried for 48 h in a vacuum over H₂SO₄ (1.45 g, 40%). The base was liberated by dissolving the salt in 2N NaOH and extracting with CHCl₃ (4 × 20 mL), washing with 10% Na₂CO₃ (10 mL) and finally with distilled H₂O. The organic phase was dried with anhydrous Na₂SO₄ and the solvent was removed under vacuum. In this way partially resolved (+)-MTA was obtained (0.69 g), [α]_D²² = +24° (*c* = 5, MeOH).

The mother liquors were concentrated to dryness and the free base was liberated from the residue (5.7 g) to afford MTA enriched in the (–) isomer (1.33 g). This base was used to prepare the (–)-*O,O'*-di-*p*-toluoyltartaric salt in MeOH (750 mL) as described above for the (+) enantiomer. The crystalline salt was collected by filtration (1.05 g, 32%) and

the free base was released as before to yield partially resolved (–)-MTA (513 mg), $[\alpha]_D^{22} = -23.4^\circ$ ($c = 5.1$, MeOH). To determine the enantiomeric excesses, Mosher's amides were prepared by reacting either the racemic mixture or each of the enriched enantiomers with (–)- α -methoxy- α -trifluoromethyl-phenylacetyl chloride²⁰ and ¹H-NMR spectra were recorded. All three amide spectra showed methyl group resonances as two doublets centered at 1.21 and 1.14 ppm ($J = 6.6$ Hz), and methoxyl singlets at 3.31 and 3.26 ppm. The integrals of these signals for both enantiomerically enriched MTA isomers allowed an ee of 90% to be calculated. MTA hydrochlorides were prepared by adding an equimolar amount of 37% HCl to a concentrated solution of each enantiomer in *i*-PrOH and subsequent addition of a few drops of Et₂O. The salts were recrystallized in *i*-PrOH to afford (+)-MTA·HCl, $[\alpha]_D^{22} = +21.5^\circ$ ($c = 2.0$, H₂O) and (–)-MTA·HCl, $[\alpha]_D^{22} = -22.0^\circ$, ($c = 2.0$, H₂O).

Enzymatic Assays

The effects of the MTA enantiomers and derivatives on MAO-A or MAO-B activities were studied using a crude rat brain mitochondrial suspension (male Sprague–Dawley rats weighing 180–220 g, sacrificed by decapitation), using 5-HT (100 μ M) and 4-dimethylaminophenethylamine (DMAPEA, 5 μ M) as selective substrates for MAO-A and –B, respectively, and detecting these compounds and their metabolites by HPLC with electrochemical detection (HPLC-ED) as described previously.¹⁴

IC₅₀ values were determined using Prism Graph Pad software, from plots of inhibition percentages (calculated in relation to a sample of the enzyme treated under the same conditions without inhibitors) versus $-\log$ inhibitor concentration. The time course of MAO inhibition by the drugs was assessed by preincubating the reaction mixture with different compounds, at appropriate concentrations, for 30 min. After preincubation, MAO activity was measured as described.^{14,21} The reversibility of the inhibitory process was assessed after repeated washing of the mitochondrial suspension, after preincubation for 10 min with drugs at appropriate concentrations. The preparations were washed three times (centrifugation and resuspension) with 0.1 M sodium phosphate buffer, pH 7.4. Finally, MAO activity was measured again, using HPLC-ED. Control samples, in which the inhibitor solution was replaced by an equal volume of water, were treated in the same way.²¹

Chromatographic Conditions

A C₁₈ reverse phase column (Macrosphere 250 mm \times 4.6 mm, Alltech, USA), an amperometric

detector (Merck-Recipe L3500A), and a chromatographic integrator (Merck-Hitachi D2500) were used to analyze the reaction mixtures. All other conditions were as previously described.¹⁴

Statistical Analysis

IC₅₀ values are given as the mean \pm SD of at least two independent experiments, each in triplicate. The statistical significance was determined using Student's *t*-test. In all cases the significance level was found to be $P < 0.05$.

RESULTS

Table I shows the structures of the compounds studied.

Chemistry

The synthesis of (\pm)-(MTA) *via* the corresponding aryl nitropropene, had been reported²² in 1963 in a low overall yield (26%), which we were able to improve to 68% (including the crystallization step to obtain the hydrochloride) using a different basic catalyst in the crucial nitroaldol condensation step. With the exception of MTA itself and its *N*-methyl derivative,^{23,24} all the molecules reported here have not been described previously. Racemic MTA, its enantiomers and all its derivatives were stored and used as hydrochloride salts, except DEMTA (as the hydrobromide) and DPMTA (as the malonate).

All the phenylisopropylamine free bases synthesized here present the characteristic chemical shifts of a 1,4-disubstituted aromatic system, resonating at 7.10 to 7.20 ppm with a coupling constant of 8.3 Hz. In the hydrochlorides this pattern is shifted downfield, presumably due to deshielding by the positively charged nitrogen atom interacting directly with the aromatic ring. In the *N*-monoalkyl-MTA series both benzylic proton resonances are shifted slightly downfield with regard to the corresponding MTA signals, suggesting that both protons lie closer to the ring plane than in MTA. On the contrary, in the *N,N*-dialkyl-MTA series the benzylic proton resonances are indicative of a more pronounced conformational change, with one of them moving downfield and the other one moving upfield. The ¹H NMR spectrum of DPMTA malonate in D₂O did not show any resonance assignable to the malonate methylene hydrogens, presumably due to rapid exchange with the solvent. In DMSO-*d*₆ the expected signal was observed at 2.85 ppm, confirming the identity of the salt.

TABLE I Structural description of MTA and derivatives, and their IC₅₀ values for MAO inhibition

Compound	R ₁	R ₂	R ₃	IC ₅₀ [μM]	
				MAO-A	MAO-B
(±)-MTA	H	H	CH ₃	0.25 ± 0.02	NE
(±)-NMMTA	H	CH ₃	CH ₃	0.89 ± 0.39	NE
(±)-DMMTA	CH ₃	CH ₃	CH ₃	2.10 ± 0.22	NE
(±)-NEMTA	H	CH ₂ CH ₃	CH ₃	1.80 ± 0.24	NE
(±)-DEMTA	CH ₂ CH ₃	CH ₂ CH ₃	CH ₃	6.45 ± 0.18	NE
(±)-NPMTA	H	CH ₂ CH ₂ CH ₃	CH ₃	2.41 ± 0.50	>10
(±)-DPMTA	CH ₂ CH ₂ CH ₃	CH ₂ CH ₂ CH ₃	CH ₃	>10	NE
(±)-NAMTA	H	CH ₂ CH=CH ₂	CH ₃	3.50 ± 1.22	>10
(±)-DAMTA	CH ₂ CH=CH ₂	CH ₂ CH=CH ₂	CH ₃	>10	NE
(±)-MTAB	H	H	CH ₂ CH ₃	0.84 ± 0.16	NE
(+)-MTA				0.11 ± 0.03	NE
(-)-MTA				2.04 ± 0.40	NE
<i>l</i> -Deprenyl				>10	0.007

The IC₅₀ values were calculated from the inhibition *vs* -log concentration curves, obtained from at least two independent experiments, with each concentration assayed in triplicate. N.E. = No Effect at 10 μM. (±)-MTA and *l*-deprenyl were used as positive controls for MAO-A and MAO-B inhibition, respectively.

MAO Inhibition

Table I summarizes the *in vitro* MAOI effects of (±)-MTA, its enantiomers, its *N*-alkyl derivatives and MTAB. (+)-MTA was the most potent MAOI-A, followed by its racemic modification which was about half as potent (Figure 1). (-)-MTA was about eighteen times less potent than its enantiomer. Most of the derivatives had no effect on MAO-B with the exception of NPMTA and NAMTA which showed weak inhibitory activity.

A representative sample of the drugs was tested regarding the reversibility of their inhibitory

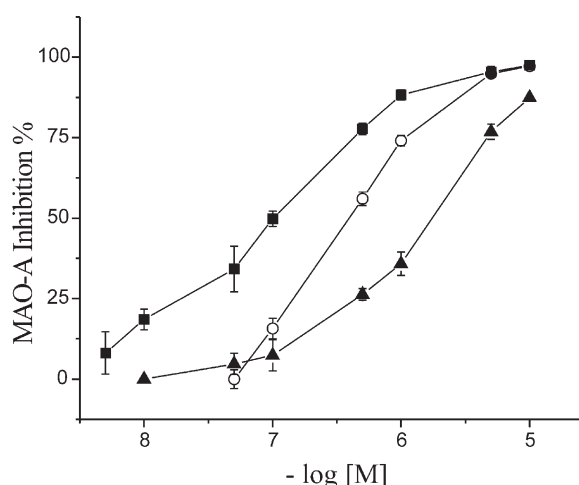


FIGURE 1 Effects of (±)-MTA (○) and its (S)(+)- (■) and (R)(-) (▲) isomers upon MAO-A. Enzyme inhibition is represented as the percentage of inhibition of deamination of 5-HT, as measured by HPLC-ED. Each point is the mean ± SD of three determinations.

properties upon MAO-A. As shown in Table II, all the compounds proved to be reversible MAOI-A, as evidenced by the recovery of the enzyme activity measured after repeated washing of the preparation.

Since the lack of effect of these compounds toward MAO-B might be due to time dependency of the inhibitory process (as occurs in the case of suicide inhibitors such as deprenyl), we evaluated the effect of incubating the enzyme with the drugs for 0 or 30 min. As shown in Table III, none of the compounds inhibited MAO-B under these conditions. As expected, the MAO-B inhibitory profile of *l*-deprenyl (used as a positive control) was time-dependent, showing significantly stronger effects after 30 minutes preincubation.

TABLE II Reversibility of MAO-A inhibition produced by some substituted derivatives of MTA, as determined by repeated washing

Compound [10 ⁻⁵ M]	Percent Inhibition	
	Before washing	After washing
NAMTA	74.2 ± 1.5	0.0 ± 8.3
NEMTA	81.4 ± 1.4	0.0 ± 8.8
DMMTA	79.7 ± 1.4	31.0 ± 17.6
MTAB	91.2 ± 1.0	21.4 ± 5.8

Crude mitochondrial suspensions were preincubated for 10 min with inhibitor and then the preparation was washed three times by centrifugation and resuspension. MAO-A activity of the preparation and of the controls was determined by HPLC-ED using 5-HT as selective substrate. Each value is the mean ± SD of triplicate determinations.

TABLE III Effect of preincubation on the inhibition of MAO-B by MTA and derivatives

Compound [10^{-5} M]	Percent inhibition of MAO-B Preincubation time (min)	
	0	30
MTA	3.8 ± 5.16	3.7 ± 6.4
NMMTA	15.6 ± 3.1	9.7 ± 5.7
DMMTA	14.4 ± 1.6	2.3 ± 4.0
NEMTA	17.8 ± 4.2	15.0 ± 5.8
DEMTA	21.2 ± 1.4	32.5 ± 4.3
NPMTA	32.9 ± 1.8	30.0 ± 5.2
DPMTA	7.1 ± 2.6	12.9 ± 4.3
NAMTA	35.6 ± 3.6	34.3 ± 9.0
DAMTA	5.8 ± 5.4	2.3 ± 4.0
MTAB	0.0 ± 2.7	5.7 ± 7.4
(-)-MTA	2.0 ± 3.4	3.0 ± 3.0
(+)-MTA	2.0 ± 3.3	13.7 ± 12.7
<i>l</i> -Deprenyl [10^{-8} M]	2.0 ± 0.2	65.3 ± 4.0*

Crude mitochondrial suspensions were preincubated at 37°C with compounds for the times indicated. Percent of MAO-B inhibition was determined by HPLC-ED. *l*-Deprenyl was used as a positive control. The values are means ± SD of triplicate determinations. * $P < 0.05$ (Student's *t*-test), compared with 0 min preincubation.

DISCUSSION

The first important finding of this work was that the presence of a substituent on the amino group of the MTA side chain decreases its activity as a MAOI-A, and that the magnitude of this effect increases with the size of the substituent. Thus, an almost linear increase of IC_{50} for MAO-A inhibition was observed when a methyl (NMMTA), an ethyl (NEMTA), a propyl (NPMTA) or an allyl (NAMTA) group was placed on the nitrogen atom. The introduction of a second alkyl substituent led to a greater increase in the IC_{50} values (as compared to the corresponding monoalkyl derivative), with the rank order of potencies being DMMTA > DEMTA > DPMTA \cong DAMTA. Replacement of the methyl group on the α -carbon of the side chain by an ethyl group (MTAB *vs* MTA) also resulted in decreased potency. These results are in agreement with previously reported data, describing the effects of *N*-substitution upon the MAOI properties of a small number of amphetamine derivatives.^{16,25,26} The most likely explanation of these results seems to be that the substituents on the amino group sterically hinder its approach to a complementary residue of the enzyme, although electronic contributions of the substituents cannot be discarded. Similar behavior, *i.e.* a decrease of MAOI-A properties, was observed after lengthening the alkyl chain bound to the α -carbon of the MTA molecule. This might also be a consequence of steric hindrance around the nitrogen atom which is presumably a key binding feature, limiting access to a pharmacophoric conformation.²⁷ These preliminary observations call for analogous experiments with derivatives bearing bulkier substituents on

the nitrogen or on the α -carbon atom. Nevertheless, it is worth pointing out that, although modifications of the MTA structure studied by us generated less active drugs as compared to the parent compound, some of the derivatives are still potent MAOI-A *i.e.* MTAB, NMMTA, DMMTA, NEMTA, NPMTA, NAMTA. As is the case of MTA, all the compounds evaluated by us proved to be reversible and very selective MAOI-A. In addition, it was demonstrated for most of these substances that the lack of effects upon MAO-B was not due to time-dependence of the inhibitory process, as happens with irreversible inhibitors.

The other noteworthy result of this study was that (+)-MTA is about 18 times more potent than (-)-MTA and is therefore twice as potent as the racemic mixture. This confirms a trend observed with several other phenylisopropylamines such as amphetamine and deprenyl,^{10,26} amiflamine and its analogs^{28,29} and methylenedioxymethamphetamine (MDMA),³⁰ showing in all cases that the (*S*)-isomers (which are always dextrorotatory) are the eutomers. It is interesting to note that the (*S*)-isomers of these amphetamine derivatives are the eutomers, both as MAOI-A and as 5-HT releasing agents, the latter acting *via* their 5-HT transporter substrate character.³¹ The present results might therefore be relevant to the dissociation of the 5-HT releasing and MAOI-A properties actions of MTA, which is desirable in the perspective of understanding the structure-activity relationships of its congeners and the possible development of antidepressants with a dual mechanism of action.

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