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Synthesis of Two Nitro Analogs of Tranylcypromine: Relations of Aromatic Substitution of Nitro Groups to MAO-Inhibitory Activity

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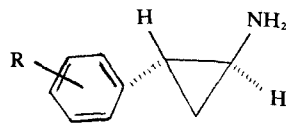
Abstract □ Two new nitro analogs of tranylcypromine, (*E*)-2-(*p*-nitrophenyl)cyclopropylamine ((*E*)-*p*-NTCP) and (*E*)-2-(*m*-nitrophenyl)cyclopropylamine ((*E*)-*m*-NTCP) were synthesized in order to examine the effect of aromatic nitro substitution on the MAO-inhibitory activity of 2-phenylcyclopropylamines. The compounds were obtained by treating *t*-butyl (*E*)-2-(*p*-nitrophenyl) cyclopropanecarbamate and *t*-butyl (*E*)-2-(*m*-nitrophenyl)cyclopropanecarbamate with *p*-toluenesulfonic acid in CH_3CN. Inhibitions of rat brain mitochondrial MAO-A and B by the compounds were examined using serotonin and benzylamine as the substrate at both *in vitro* and *ex vivo* levels. It was found from *in vitro* measurements that (*E*)-*p*-NTCP at 6.0×10^{-5}M elicited merely 22.5% inhibition against MAO-B without any effect on MAO-A. In contrast, (*E*)-*m*-NTCP showed fair degrees of inhibitions of MAO-A and B with IC_{50} values, 2.5×10^{-7}M and 1.4×10^{-6}M, respectively. It was also noted from (*E*)-*m*-NTCP that *m*-nitro substitution caused a shift of selectivity of the inhibition toward MAO-A. According to *ex vivo* measurements at 1.5, 3, 6, and 12 hr following the administration of a dose of 0.015 mmol/kg, i.p. to the rats, the inhibition percents of MAO-A by (*E*)-*m*-NTCP were 58.6, 63.7 63.6, and 46.6%, slightly lower than those observed by tranylcypromine. Whereas, (*E*)-*p*-NTCP at the same dose level did not show significant inhibitions against both MAO-A and MAO-B. Possible reasons for the difference in potencies between (*E*)-*m*-NTCP and (*E*)-*p*-NTCP were sought in relation to differing electron withdrawing effects of *m*- and *p*-substituents which will influence electron density of the side chain amino functions and the partitions.

Keywords □ 2-Phenylcyclopropylamines, (*E*)-2-(*p*-nitrophenyl)cyclopropylamine, (*E*)-2-(*m*-nitrophenyl) cyclopropylamine, Synthesis, Rat brain mitochondrial MAO, MAO inhibition potency, MAO inhibition selectivity, Structure-activity relations.

A series of 2-substituted cyclopropylamines were synthesized by Burger and Yost[1] and Kaiser *et al.*[2] and their MAO-inhibitory properties were examined by Zirkle *et al.*[3] Among the compounds, (*E*)-2-phenylcyclopropylamine (**1**, tranylcypromine, (*E*)-TCP) is an antidepressant clinically available and known to be nonspecific inhibitor toward multiple forms of MAO.[4]



R = H (**1**, (*E*)-TCP)
m-NO_2 (**2**, (*E*)-*m*-NTCP)
p-NO_2 (**3**, (*E*)-*p*-NTCP)

Tranylcypromine has been recently established to be a suicide inhibitor of MAO by Silverman[5] mainly on the basis of the isolation of cinnamaldehyde from drug-enzyme complex. Analogous suicide inhibitions were also found among other cyclopropylamines such as N-(1-methylcyclopropyl)benzylamine,[6] N-cyclopropylbenzylamine,[7] and 1-phenylcyclopropylamine.[8]

Despite such mechanistic studies, it was noted that more studies are needed regarding how 2-phenylcyclopropylamines interact with the active sites of MAO possibly by the method of structure-activity relationships. For this, it may require ultimately to isolate initial reversible interactions from the subsequent irreversible ones since overall inhibitory potencies be determined by both.[9] Such interaction studies appeared especially essential to the topographic understanding of MAO and to the separate characterization of MAO-A and MAO-B as well as in order to design more potent and selective inhibitors than expected by tranylcypromine.

A few reports on the same line have appeared in literatures. One by Zirkle *et al.*[3] attempted to

describe a mode of interaction of 2-phenylcyclopropylamines with MAO by using *in vivo* inhibition data of stereoisomers of 2-phenylcyclopropylamine and its 1-methyl analog. Recently, we have reexamined their analysis by using *in vitro* data of synthesized pure compounds.[10] A report by Belleau and Moran[11] described an interaction of 2-phenylcyclopropylamine with MAO through the cyclopropane ring which is rich in electron density. A quantitative structure-activity relationships by Fujita[12] did not provide any conclusive account for the interaction.

Accordingly, we designed to synthesize a series of 2-phenylcyclopropylamines in order to determine their modes of interactions with MAO and their structural requirements for selectivity and potency. Among the compounds, we report in this paper synthesis of two new nitro analogs, (*E*)-2-(*m*-nitrophenyl)cyclopropylamine (**2**, (*E*)-*m*-NTCP) and (*E*)-2-(*p*-nitrophenyl)cyclopropylamine (**3**, (*E*)-*p*-NTCP) and their MAO-inhibitory properties *in vitro* and *ex vivo*.

EXPERIMENTAL METHODS

Materials and animals

Tranylcypromine sulfate was a kind gift of Smith Kline & French Laboratories, U.S.A. from which authentic hydrochloride salt was prepared. *Meta*-and *para*-nitrostyrenes were purchased from Fairfield Chemical Co., Inc., Blythewood, S.C., U.S.A.. (*E*)-2-phenylcyclopropanecarboxylic acid, 9-anthracenemethanol, ethanethiol, sodium hydride, glycine ethyl ester hydrochloride, and Diazald were obtained from Aldrich Chemical Co., Inc., U.S.A.. Serotonin-creatinin sulfate complex, benzylamine hydrochloride, and bovine albumin were purchased from Sigma Chemical Co., St. Louis, U.S.A.. A 3% OV-17 80/100 mesh Chromosorb W (HP) was obtained from Supelco Inc., U.S.A. and Silica gel 60A (PE SILG) of Whatman Ltd. was used for TLC. All other chemicals and solvents were obtained from a local market and were of reagent grade.

Male Sprague-Dawley rats (150-200g) were accommodated in a controlled animal room for at least 2 weeks prior to use and given food (Samyang animals food) and water *ad libitum*.

Instrumentation

Melting points were determined on a Sybron Thermolyne (Olympus, Tokyo) and are uncorrected. A Shimadzu model 435 infrared spectrometer was used to take IR spectra. NMR spectra were obtained with a Varian EM-360A or a Varian EM-360L 60 MHz spectrometer. UV/VIS absorptions were recorded using a Hitachi model 200-20 UV-VIS spectrophotometer. A Hewlett Packard model HP 5985B GC/MS system was used to collect direct probe mass spectral data. Electron ionization voltage was 70 eV. Gas chromatography was performed on a Hitachi model 163 gas chromatograph equipped with a hydrogen flame detector. A glass column (2.0m × 3mm i.d.) packed with 3% OV-17 on 80/100 mesh Chromosorb W (HP) was used with carrier gas (N_2) at 50 m*l* $min.^{-1}$ General operating conditions were specified on each section. A Sorvall superspeed refrigerated centrifuge, RC-2B, Sorvall Inc., U.S.A. and motor-driven glass homogenizer (Potter Elvehjem type) with a Teflon resin pestle were used. Elementary analyses were performed by Analytical Research Section of Dong-A Pharm. Co., Ltd., Seoul.

Synthesis of (E)-2-(m-nitrophenyl)cyclopropanecarboxylic acid (5)

Ethyl diazoacetate (4.47g, 0.0392 mol) was condensed with *m*-nitrostyrene (4.47g, 0.03mol) as described[13] and 5.55g (78.7%) of ethyl 2-(*m*-nitrophenyl)cyclopropanecarboxylate (**4**) was obtained after the distillation (bp 175-180°C/6 mmHg). IR (neat) 1720cm^{-1} (ester C=O), 1520 & 1340 ($-NO_2$). **4** was determined to be a mixture of (*E*)/(*Z*)-isomers (1.9:1) by GC (column temperature 150-280°C, programmed at 20°C min^{-1}) with t_Rs, 5.7 min for (*E*)-isomer and 5.5 min for (*Z*)-isomer. Each peak was separately assigned by referring to the result of selective hydrolysis.

4 (5.55g, 0.0236 mol) was refluxed with NaOH (0.66g, 0.0165 mol) in 75% EtOH (15 m*l*) for 7 hr. A work up gave (*E*)-2-(*m*-nitrophenyl)cyclopropanecarboxylic acid (**5**) in a yield of 1.95g (40%) after two recrystallizations from hot H_2O. mp 154-155°C (lit.[13] 155.5-156.5°C). IR(KBr) 3100-2900cm^{-1} (acid OH), 1680 (C=O), 1520 & 1340 ($-NO_2$). NMR (DMSO-d_6) δ 8.3-7.9 (m, 2H, aromatic H), 7.9-7.4 (m, 2H, aromatic H), 2.8-2.25 (m, 1H, cyclopropyl H), 2.25-1.8 (m, 1H, cyclopropyl H), 1.8-1.3 (m, 2H, cyclopropyl H), 12.5 (bs, 1H, carboxyl H). Purity of the synthesized **5** was determined by GC (column temperature, 150-280°C, programmed at 20°C min^{-1}) following the methylation with CH_2N_2. One peak at t_R 5.40 min was observed for **5** methyl ester.

(*Z*)-2-(*m*-nitrophenyl)cyclopropanecarboxylic acid (**6**) was isolated by further hydrolysis of a remaining mixture after the work up of **5**. Yield 0.66g (13.5%) after the recrystallization from to-

luene-hexane. mp 177-178 °C (lit.[14] 178.7-179.7 °C). IR(KBr) 3100-2900cm^{-1} (acid OH), 1690 (acid C=O), 1520 & 1340 ($-NO_2$). NMR (DMSO-d_6) δ 8.4-7.1 (m, 4H, aromatic H), 3.1-2.4 (m, 1H, cyclopropyl H), 2.4-1.85 (m, 1H, cyclopropyl H), 1.85-1.0 (m, 2H, cyclopropyl H), 12.1 (bs, 1H, carboxyl H). GC of **6** methyl ester at the same analysis conditions as for **5** methyl ester showed one peak at t_R 5.10 min.

Synthesis of t-butyl (E)-2-(m-nitrophenyl)cyclopropanecarbamate **(10)**

5 (0.621g, 3 mmole) was refluxed with $SOCl_2$ (2.5g 0.021 mol) and evaporation of the excess $SOCl_2$ under reduced pressure gave (*E*)-2-(*m*-nitrophenyl) cyclopropanecarbonyl chloride (**7**). IR(neat) 1770 cm^{-1} (acid chloride C=O). **7** was converted to (*E*)-2-(*m*-nitrophenyl)cyclopropanecarboxazide (**8**) and then (*E*)-2-(*m*-nitrophenyl) cyclopropylisocyanate (**9**) employing a wet sodium azide method.[2] IR(neat) of **8** 2100 cm^{-1} ($-N_3$), 1690-1700 (C=O). Yield of **9** 0.53g (86.7%). IR(neat) 2250 cm^{-1} (−NCO), no absorption at 2100 cm^{-1} (azide). **9** (0.52g, 2.5 mmole) was refluxed with *t*-BuOH (15m*l*) for 2 hr. Following the evaporation of excess *t*-BuOH, *t*-butyl (*E*)-2-(*m*-nitrophenyl)cyclopropanecarbamate (**10**) was obtained. Yield 0.49g (71%) after two recrystallizations from benzene and hexane. mp 102 °C. IR(KBr) 3350 cm^{-1} (NH), 1680 (amide I), 1520 (amide II & $-NO_2$), 1340 ($-NO_2$). NMR ($CDCl_3$) δ 8.35-7.85 (m, 2H, aromatic H), 7.75-7.25 (m, 2H, aromatic H), 4.95 (bs, 1H, NH), 3.15-2.4 (m, 1H, cyclopropyl H), 2.4-1.85 (m, 1H, cyclopropyl H), 1.75-0.9 (m, 11H, cyclopropyl H & *t*-butyl H). TLC (EtOAc /MeOH, 95:5, ninhydrin coloring) R_f 0.68.

Synthesis of (E)-2-(m-nitrophenyl)cyclopropylamine **(2, (E)-m-NTCP)**

The carbamate, **10** (0.33g, 1.2 mmole) was dissolved in CH_3CN (6m*l*) to which *p*-toluenesulfonic acid H_2O (0.456g, 2.4 mmole) in CH_3CN (6m*l*) was added. Following the stirring at room temperature for 1 hr, the formed precipitate of **2** *p*-toluenesulfonate was filtered and washed with anhydrous ether. Yield 0.37g (88.1%). mp 193-194 °C. IR (KBr) 3000 cm^{-1} ($-NH_3^+$), 1520 & 1340 ($-NO_2$), 1190-1120 ($-SO_3^-$). NMR (DMSO-d_6) δ 8.16 (bs, 3H, NH_3^+, exchanged with D_2O), 7.86 (d, 2H, nitrophenyl H), 7.48 (d, 2H, nitrophenyl H), 7.35 (d, 2H, tolyl benzene H), 6.96 (d, 2H, tolyl benzene H), 3.2-2.7 (m, 1H, cyclopropyl H), 2.6-2.3, (m, 1H, cyclopropyl H), 2.25 (s, 3H, tolyl methyl H), 1.6-1.1 (m, 2H, cyclopropyl H).

2 *p*-toluenesulfonate dissolved in H_2O was made alkaline with Na_2CO_3 followed by the extraction with ether. The ether solution was added to excess oxalic acid in ether to obtain **2** oxalate. mp 187-189 °C after the recrystallization from EtOH/MeOH. IR(KBr) 3000 cm^{-1} ($-NH_3^+$), 1520 & 1340 ($-NO_2$), 1710 (acid C=O). NMR (DMSO-d_6) δ 7.86 (d, 2H, aromatic H), 7.5 (bs, 5H, aromatic H & $-NH_3^+$, aromatic two protons appear at δ 7.43 as a doublet after the exchange with D_2O), 3.05-2.6 (m, 1H, cyclopropyl H), 2.6-2.25 (m, 1H, cyclopropyl H), 1.7-1.0 (m, 2H, cyclopropyl H). MS, *m/z* (relative intensity) 178(22, M^+), 161 (27.3), 130 (85.8), 115 (27.3) 103(27.3), 56(base peak). Anal. Calcd. for $C_{11}H_{12}N_2O_6$: C, 49.25; H, 4.48; N, 10.45. Found: C, 49.13; H, 4.53; N, 10.49.

Synthesis of (E)-2-(p-nitrophenyl) cyclopropanecarboxylic acid **(11)**

(*E*)-2-phenylcyclopropanecarboxylic acid (4.86g, 0.03 mol) was treated with HNO_3 as described[15] to obtain (*E*)-2-(*p*-nitrophenyl)cyclopropanecarboxylic acid (**11**). Two recrystallizations from xylene gave a pale yellow plate. Yield 2.64g (42.5%). mp 197-200 °C (lit.[15] 197-199 °C). IR(KBr) 1680 cm^{-1} (acid C=O), 1510 & 1340 ($-NO_2$). NMR (DMSO-d_6) δ 8.25 (d, 2H, aromatic H), 7.54 (d, 2H, aromatic H), 2.85-2.3 (m, 1H, cyclopropyl H), 2.25-1.8 (m, 1H, cyclopropyl H), 1.8-1.2 (m, 2H, cyclopropyl H), 12.6 (bs, 1H, carboxyl H). TLC (benzene/MeOH, 95:5, glucose/aniline coloring) R_f 0.16.

Synthesis of t-butyl (E)-2-(p-nitrophenyl) cyclopropanecarbamate **(15)**

11 (2.07g, 0.01 mol) was converted to (*E*)-2-(*p*-nitrophenyl)cyclopropylisocyanate (**14**) similarly to the synthesis of **9** from **5**. (*E*)-2-(*p*-nitrophenyl) cyclopropanecarbonyl chloride (**12**); mp 65-67 °C. IR(KBr) 1770cm^{-1} (acid chloride C=O), 1510 & 1340 ($-NO_2$). (*E*)-2-(*p*-nitrophenyl)cyclopropanecarboxazide (**13**); mp 87-89 °C. IR(KBr) 2160cm^{-1} ($-N_3$), 1700 (C=O). Yield of **14** 1.92g (94.1%). mp 55-56 °C. IR(KBr) 2260 cm^{-1} (−NCO), 1510 & 1340($-NO_2$). The isocyanate, **14** (1.90g, 0.0093 mol) was mixed with *t*-butyl alcohol (40 m*l*) and refluxed for 2 hr. Excess *t*-BuOH was evaporated to obtain *t*-butyl (*E*)-2-(*p*-nitrophenyl) cyclopropanecarbamate (**15**) as a white solid. Yield 1.6g (61.9%) after the recrystallization from EtOAc. mp 155-156 °C. IR(KBr) 3350 cm^{-1} (NH), 1680 (amide I), 1510 (amide II & $-NO_2$), 1340 ($-NO_2$). NMR($CDCl_3$) δ 8.22 (d, 2H, aromatic H), 7.33 (d,

2H, aromatic H), 5.06 (bs, 1H, NH), 3.05-2.6 (m, 1H, cyclopropyl H), 2.5-1.9 (m, 1H, cyclopropyl H), 1.7-1.1 (m, 11H, cyclopropyl H & *t*-butyl H). TLC (EtOAc/MeOH, 95:5, ninhydrin coloring) R_f 0.69.

Synthesis of (E)-2-(p-nitrophenyl)cyclopropylamine (3, (E)-p-NTCP)

The *p*-nitro carbamate, **15** (1.25g, 0.0045 mol) was dissolved in CH_3CN (60m*l*), to which was added dropwise *p*-toluenesulfonic acid H_2O (1.71g, 0.009 mol) dissolved in CH_3CN (25m*l*) and the mixture stirred at room temperature for 1 hr. The precipitate was filtered and washed with CH_3CN and then with anhydrous ether. Yield of (*E*)-2-(*p*-nitrophenyl)cyclopropylamine *p*-toluenesulfonate (**3** *p*-toluenesulfonate) was 1.36g (86.6%). mp > 300°C. IR(KBr) 3000 cm^{-1}(NH_3^+), 1520 & 1340 ($-NO_2$), 1210-1120 ($-SO_3^-$). NMR (DMSO-d_6) δ 8.3-7.5 (m, 5H, aromatic H & $-NH_3^+$, ammonium protons exchanged with D_2O), 7.5-6.7 (m, 6H, aromatic H), 3.1-2.6 (m, 1H, cyclopropyl H), 2.6-2.3 (m, 1H, cyclopropyl H), 2.26 (s, 3H, tolyl methyl H), 1.85-1.1 (m, 2H, cyclopropyl H). MS, *m/z* (relative intensity) 178 (40.9, M^+), 172(55.4, from p-TsOH), 130(48.1), 115(35.4), 107(46.9), 91(base peak, from p-TsOH). Anal. Calcd. for $C_{16}H_{18}N_2O_5S$: C, 54.86; H, 5.14; N, 8.00. Found: C, 54.60; H, 5.15; N, 7.88.

MAO inhibition studies

Activity of MAO-A was measured using serotonin as a substrate by the method of Sjoerdsma *et al.*[16] described for the metabolism of serotonin and by UV method of Udenfriend *et al.*[17] Specific procedures to determine IC_{50} values as well as the preparation of rat brain mitochondrial MAO suitable to the present laboratory condition were described previously.[18] Activity of MAO-B was measured using benzylamine as a substrate according to Tabor *et al.*[19] with modifications. Optimum conditions for the measurement of activity of MAO-B and IC_{50} values were described in the previous report.[10] The mitochondrial protein content was determined according to Lowry *et al.*[20] with bovine albumin as the standard.

For *in vitro* experiments, (*E*)-*p*-NTCP *p*-toluenesulfonate (7.0mg) was dissolved in EtOH (1m*l*) and glycerine (0.5 m*l*) and diluted to 100 m*l* with H_2O to obtain a stock solution (2×10^{-4}M), from which further dilutions were made with H_2O for desired concentrations. Experiments to examine the effect of the solvents on MAO inhibition were performed and the solvents proved not to affect the results. (*E*)-*m*-NTCP oxalate and (*E*)-TCP HCl were dissolved in H_2O and used.

For *ex vivo* experiments, (*E*)-*p*-NTCP *p*-toluenesulfonate (10.5mg) was dissolved in EtOH (1m*l*) and glycerine (2m*l*) and made to 10m*l* with H_2O. (*E*)-*m*-NTCP oxalate (6.03mg) was dissolved in EtOH (0.7m*l*) and glycerine (1.4m*l*) and made to 7m*l* with H_2O. (*E*)-TCP HCl was dissolved in H_2O. A dose corresponding to 0.015 mmole/kg was administered to each of 2 rats in a volume of ~0.5m*l* by intraperitoneal injection. After various times of 1.5, 3, 6, and 12hr, rat brains were removed and the MAO activities were measured. Controls were prepared using the enzyme from the rats administered vehicles only.

RESULTS AND DISCUSSION

Synthesis of aromatic nitro analogs of tranylcypromine

(*E*)-2-(*m*-nitrophenyl) cyclopropanecarboxylic acid and its (*Z*)-isomer were obtained by condensing *m*-nitrostyrene with ethyl diazoacetate followed by the selective hydrolysis.[13] The condensation mixture consisted of esters of (*E*)/(*Z*)-isomers (1.9:1) as determined by GC and by referring to the result of selective hydrolysis. Similarly, the condensation of *p*-nitrostyrene with ethyl diazoacetate provided a mixture of esters of (*E*)/(*Z*)-isomers (1.8:1). The GC (150-280°C, programmed at 20°C min^{-1}) gave two peaks at t_R 5.9 min for (*E*)-isomer and t_R 5.6 min for (*Z*)-isomer. (*E*)-2-(*p*-nitrophenyl)cyclopropanecarboxylic acid was however synthesized by direct nitration of (*E*)-2-phenylcyclopropanecarboxylice acid,[15] since by the method, pure material was more easily obtainable.

It was initially attempted to synthesize (*E*)-2-(*p*-nitrophenyl)cyclopropylamine by acid or base hydrolysis of (*E*)-2-(*p*-nitrophenyl)cyclopropylisocyanate. The resultant mixture from either hydrolysis was a black colored material denoting severe decompositions so that isolation of the desired product was not possible. A literature by Erhardt[21] has discussed a similar decomposition observed on the acid hydrolysis of (*E*)-2-(3,4-dibenzyloxyphenyl)cyclopropylisocyanate and either catalytic hydrogenolysis of benzylcarbamate[21] or deblocking of 9-anthrylmethyl carbamate under neutral condition[22,23] was used to prepare 2-(3,4-dihydroxyphenyl)cyclopropylamine. Hence, for the purpose, 9-anthrylmethyl (*E*)-2-phenylcyclopropanecarbamate (mp 182-183°C, recrystallized from toluene) and 9-

anthrylmethyl (*E*)-2-(*p*-nitrophenyl)cyclopropanecarbamate (mp 208-210 °C, recrystallized from toluene) were prepared. When 9-anthrylmethyl (*E*)-2-phenylcyclopropanecarbamate was reacted with sodium ethanethiol in DMF for 2 hr at 25 °C, it was possible to identify the formation of (*E*)-2-phenylcyclopropylamine by TLC and by IR of its hydrochloride salt. However, in case of the 9-anthrylmethyl (*E*)-2-(*p*-nitrophenyl)cyclopropanecarbamate, only a severe charring mixture was produced all under varying temperatures and reaction times without any positive identification of an amine.

In another experiment, *t*-butyl (*E*)-2-(*p*-nitrophenyl)cyclopropanecarbamate was treated with anhydrous EtOH-HCl. This anhydrous procedure also gave a dark black colored mixture which was manifesting unstable nature of the product. Therefore, experiments were designed to treat the *p*-nitro carbamate as well as *t*-butyl (*E*)-2-phenylcyclopropanecarbamate and *m*-nitro carbamate with *p*-toluenesulfonic acid because the method employing *p*-toluenesulfonic acid was able to remove *t*-butyloxycarbonyl protecting group in mild conditions of the presence of *t*-butyl and *p*-methoxybenzyl esters.[24] When the reaction was performed in EtOH/ether solvent under stirring the mixture at room temperature for 20hr, the yield of *p*-toluenesulfonate of each amine was found to be 89% for underivatized, 62% for *m*-NO_2, and less than 7% for *p*-NO_2. The reaction mixture of *p*-nitro carbamate was colored at the end of the reaction and a starting material was observed by TLC. The C-N cleavage of the carbamate by acid catalysis is known to occur through the N-H protonation.[25] Therefore, the low yield of *p*-nitro compound appears to result from the difficulty in that process because of electron withdrawing resonance effect of the *p*-nitro group.

Recently Yamada *et al.*[26] reported that acetonitrile is an effective solvent to remove 4-methoxybenzyloxycarbonyl group with *p*-toluenesulfonic acid. When acetonitrile was used for reactions of underivatized and two nitro carbamates, the reactions were all found to complete in 1hr and yields of the deprotected products were over 87%. Attempts to recrystallize (*E*)-2-(*p*-nitrophenyl)cyclopropylamine *p*-toluenesulfonate as well as to convert it to other salt forms all failed because of unstable nature of the compound. As an example, it was noted even while taking NMR spectrum in DMSO by its fast color change to untransparent dark brown. Therefore, the compound was directly used for the study of MAO inhibitions.

MAO inhibition studies

1. In vitro and ex vivo inhibitions

Table I represents *in vitro* inhibitions of rat brain mitochondrial MAO-A and B by (*E*)-TCP and its nitro analogs. The inhibition of MAO-A by (*E*)-*m*-NTCP was found to be comparable to that by (*E*)-TCP. On the other hand, IC_{50} for the inhibition of MAO-B by (*E*)-*m*-NTCP was 1.4×10^{-6}M which was 0.06 times as potent as (*E*)-TCP. The result also indicated that *m*-nitro substitution at the benzene ring of (*E*)-TCP produced a shift in selectivity of the inhibition toward MAO-A as noted by A/B ratio of 5.6:1. According to this experiment as well as previous result,[10] (*E*)-TCP was more potent against MAO-B with A/B ratio being 1:3.7.

Inhibition of either MAO-A or B by (*E*)-*p*-NTCP in its *p*-toluenesulfonate salt was found to be very low so that at 6×10^{-5}M significant inhibitions were not observed.

Results of *ex vivo* studies were summarized in Table II. It was found that the inhibitions by (*E*)-*m*-NTCP were slightly lower than those by (*E*)-*TCP* over 12 hr. (*E*)-*p*-NTCP did not show any inhibitions against both MAO-A and MAO-B.

Table I. ***In vitro*** **inhibitions of rat brain mitochondrial MAO-A and B by tranylcypromine and its aromatic nitro analogs.**

Inhibitor[1]	MAO-A[2]		MAO-B[3]		Selectivity (A/B)
	IC_{50}(M)	Relative potency	IC_{50}(M)	Relative potency	
(*E*)-TCP	2.9×10^{-7}	1.0	7.8×10^{-8}	1.0	1/3.7
(*E*)-*m*-NTCP	2.5×10^{-7}	1.2	1.4×10^{-6}	0.06	5.6/1
(*E*)-*p*-NTCP (6×10^{-5}M)	no inhibition		22.5% inhibition		

[1](*E*)-TCP, (*E*)-2-phenylcyclopropylamine (HCl); (*E*)-*m*-NTCP, (*E*)-2-(*m*-nitrophenyl)cyclopropylamine (oxalate); (*E*)-*p*-NTCP, (*E*)-2-(*p*-nitrophenyl)cyclopropylamine (*p*-toluenesulfonate). [2]Measured according to Sjoerdsma *et al.*[16] using serotonin as a substrate. [3]Measured by the method of Tabor *et al.*[19] using benzylamine as a substrate.

Table II. ***Ex vivo*** **effects of tranylcypromine and its aromatic nitro analogs on activity of MAO-A in rat brain mitochondria.**

Inhibitor[1]	Dose (mmole/kg i.p.)	% Inhibition at various times(hr) following the injection[1]			
		1.5	3	6	12
(*E*)-TCP	0.015	86.9	84.5	82.7	62.6
(*E*)-*m*-NTCP	0.015	58.6	63.7	63.6	46.6
(*E*)-*p*-NTCP	0.015	no inhibition[2]			

[1]Refer to Table I for measurements and abbreviations.
[2]The same result was found when MAO-B was measured.

2. *Structure-Activity Relations*

A primary consideration of the synthesis of nitro analogs was made in view of quantitative structure-activity relations reported previously for 2-phenylcyclopropylamines having substituents at the phenyl ring. The equation derived by Fujita[12] using *in vivo* data was:

$$\text{Log } 1/C = -0.746\pi + 1.858\ \sigma_2 + 0.502Es^3 + 5.180 \quad \text{(Eq. 1)}$$

The equation indicates that *in vivo* potency of 2-phenylcyclopropylamines is negatively dependent on a partition and that *meta* substitution is unfavorable to the potency. Positive dependence on σ_2 was indicative of the charge transfer type interactions. Kang and Choi[18] considered metabolic factors and derived two separate equations for *p*-substituent compounds (Eq. 2) and for the compounds without *p*-substituents (Eq. 3) as follows:

$$\text{Log } 1/C = 1.62\ \sigma - 1.31\ \pi + 6.48 \quad (r = 0.992) \quad \text{(Eq. 2)}$$

$$\text{Log } 1/C = 1.10\ \sigma - 1.91\ \pi + 6.20 \quad (r = 0.922) \quad \text{(Eq. 3)}$$

Table III. Anticipating inhibitory potencies of aromatic *p*- and *m*-nitro analogs of tranylcypromine.

Substituent	Eq. 1[1]		Eq. 2,3[1]	
	Log 1/C	Relative potency	Log 1/C	Relative potency
H[2]	5.80	1	6.20	1
p-NO_2[3]	7.15	22.3	7.80	39.4
m-NO_2[3]	5.99	1.55	7.00	6.3

[1]See the text. [2]Observed value of log 1/C for tranylcypromine was 5.96 by Zirkle *et al.*[3] [3] π values (*p*, −0.04; *m*, −0.01) were taken from Fujita *et al.*[34], σ (*p*, 0.78; *m*, 0.71) from Hansch *et al.*[28], and Es^3 (*p*, 1.24; *m*, −1.28) from Kutter and Hansch.[38]

It can be deduced by equations that electron withdrawing groups in both series of compounds contributed positively to the potency, possibly owing to their effects on side chain amino groups, which are considered to be important in terms of their interactions with active sites of MAO. The effect of a hydrophobicity was similar to that found in eq. 1. The anticipating inhibition potencies of the nitro analogs were calculated as shown in Table III. All were expected to be more potent than (*E*)-TCP.

The results we obtained in the present study were however found to deviate seriously from the expected values especially for *p*-nitro analog, (*E*)-*p*-NTCP. In the first place, this was at least indicative of the fact that electronic effects offered by the nitro substituents do not reside in the aromatic ring, contrary to the suggestion by Fujita[12] and Johnson,[27] but influence reactivity of the side chain nitrogen functions.

The σ values we adopted for nitro substituents were the ones of the standard Hammett constants.[28] However, from the experimental result, we also found a necessity to reexamine suitability of the constants for 2-phenylcyclopropylamines. The cyclopropane ring is known to be typical in its higher electronic transmission capability compared to alkyl chain[14,29] and the effect might be reflected on lower pKa value of 2-phenylcyclopropanecarboxylic acid (pKa = 4.570) compared to that of 3-phenylpropanoic acid (pKa = 4.709).[30] In amino group compounds much lower pKa values can be expected due to direct participation of amino electrons in the conjugation. In fact, pKa values of (*E*)-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine[31] and (*E*)-2-phenylcyclopropylamine[32] were 8.11 and 8.13, respectively and about 1.7 unit lower than for phenylethylamine (pKa = 9.88) and amphetamine (pKa = 9.90).[33] Although the difference in pKa values between 2-phenylcyclopropanecarboxylic acid and its nitro analogs was about 0.4 unit without much difference depending on the substituent positions,[14] nitro substituent effects on pKa of 2-phenylcyclopropylamines will be much greater and the positional effect will be conspicuous.

The π values for the analysis were taken from the data of substituted phenylacetic acids.[34] However, it is most likely that in case of 2-phenylcyclopropylamines, the amino group being affected by the electron withdrawing effects, the π values be much higher than −0.01 for *m*-NO_2 and −0.04 for *p*-NO_2.

With the σ and π assumptions described

above, it may be deduced that the difference in *in vivo* potencies between (*E*)-*p*-NTCP and (*E*)-*m*-NTCP resulted from the differing electronic effect. In case of (*E*)-*p*-NTCP, the electron density on the amino group will be extremely lowered so that nucleophilic attack to the active site of MAO becomes very unfavorable. In this respect, Martin *et al.*[35] has reported that the potency of propynylamines is parabolically related to pKa with optimal pKa being 6.2. In addition, higher π values should have used for both *p*-nitro and *m*-nitro substituents, which ought to lead to the lowering of potencies to larger degrees than expected in Table III. It might be assumed that the pKa of (*E*)-*m*-NTCP is within the optimal range.

Regarding *in vitro* potencies of two nitro analogs, it appears likely that as for propargylamines[36] high π values may be needed for increasing potencies because hydrophobicity can contribute to the penetration to MAO. There is an argument whether increasing π values can induce selectivity toward higher inhibition of MAO-A which is surrounded by more hydrophobic environment compared to MAO-B.[37] But the shift in the selectivity observed by *m*-nitro substitution might be explained by such an assumption. Increasing π values induced by *m*- and *p*-nitro substitutions will be all favorable to the inhibition of MAO-A and to less extent to that of MAO-B but decrease in electron density on the amino group of *p*-nitro compound should be detrimental to the potency as reflected by the fact that (*E*)-*p*-NTCP did not exhibit significant inhibition at 6×10^{-5}M while *in vitro* potency of (*E*)-*m*-NTCP is comparable to that of (*E*)-TCP. In order to help prove the assumptions made in the study, we are obtaining pKa and partition values of the synthesized nitro analogs. We are also determining whether the low potency of *p*-nitro analog results from its unstable nature.

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