Novel Monoamine Oxidase Inhibitors, 3-(2-Aminoethoxy)-1,2-benzisoxazole Derivatives, and Their Differential Reversibility

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ABSTRACT—Although possible usefulness of non-selective monoamine oxidase (MAO) inhibitors for Parkinson’s disease therapy has been suggested in the literature, MAO inhibitors whose inhibition is reversible and have dual action to both MAO-A and -B subtypes is not available yet. Subtype selectivity and reversibility of a series of novel MAO inhibitors, 3-(2-aminoethoxy)-1,2-benzisoxazole derivatives, were studied. Several dual MAO inhibitors, which inhibit both MAO-A and -B, were obtained. When administered to mice, their effects were generally reversible. Among the derivatives, RS-1636 and RS-1653 had much longer duration of brain MAO-B inhibition than that of MAO-A. In vitro, the inhibited MAO-A activity by these compounds was partially recovered by buffer change at 4°C, while little MAO-B activity was recovered. Although it is not fully elucidated yet, the reversibility of these inhibitors is probably determined primarily by this dissociation profile. This unique differential reversibility indicates that optimization of the balance of actions can be achieved by differentiating reversibility to each target molecule.

Keywords: Monoamine oxidase, Tight-binding inhibitor, Reversibility, Parkinson’s disease
(Shizuoka) were used to obtain brain mitochondria, following our previously reported method (6, 7). Animals were housed in our temperature- and humidity-controlled animal quarters with free access to food and water for more than 1 week before use. All experiments were carried out in accordance with the guidelines provided by the Institutional Animal Care and Use Committee of Sankyo Co., Ltd. (Tokyo).

Chemicals

\[^{14}C\]5-Hydroxytryptamine creatinine sulphate (5-HT) (55 mCi/mmol) and \[^{14}C\]2-phenylethylamine hydrochloride (PEA) (56 mCi/mmol) were obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). 5-HT, tranylcypromine hydrochloride and PEA were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Aldrich (Milwaukee, WI, USA) and Tokyo Kasei (Tokyo), respectively. l-Deprenyl hydrochloride and clorgyline hydrochloride were purchased from Research Biochemicals, Inc. (Natick, MA, USA). RS-8359 ((±)-4-(cyanoanilino)-7-hydroxycyclopenta[3,2-e]pyrimidine) and moclobemide were synthesized by Ube Kosan Co., Ltd. (Yamaguchi) and the Institute of Science and Technology, Inc. (Tokyo), respectively. Reserpine solution (Apoplon®) was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo). Novel 3-(2-aminoethoxy)-1,2-benzisoxazole derivatives and lazabemide were synthesized by Ube Kosan Co., Ltd. (Yamaguchi) and the Institute of Science and Technology, Inc. (Tokyo), respectively. Reserpine solution (Apoplon®) was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo). Novel 3-(2-aminoethoxy)-1,2-benzisoxazole derivatives and lazabemide were synthesized by Ube Kosan Co., Ltd. (Yamaguchi) and the Institute of Science and Technology, Inc. (Tokyo), respectively. Reserpine solution (Apoplon®) was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo).

Radiochemical assay of MAO activity

MAO activity was assayed as described previously (6, 7, 11). Pooled whole brains of ddY mice were homogenized in tenfold volume of cold 0.32 M sucrose and 10 mM sodium phosphate buffer (pH 7.4) with a teflon-glass homogenizer. The mitochondrial fraction was obtained by sodium phosphate buffer (pH 7.4) with a teflon-glass homogenizer. The mitochondrial fraction was obtained by sodium phosphate buffer (pH 7.4) with various concentrations of test compounds were pre-incubated in a water bath at 38°C for 20 min. Optimum substrate concentration for selective and sensitive MAO assay was determined in our previous experiments (6). Then radiolabeled substrate, \[^{14}C\]5-HT (final concentration of 0.01 μM and radioactivity of 100 mCi/mm) or \[^{14}C\]PEA (final concentration of 20 μM and radioactivity of 25 mCi/mm), was added to the tubes, and incubation was continued for another 20 min. After the reaction was stopped with HCl, the labeled metabolites were extracted into ethyl acetate-toluene (1:1 V/V) by vigorous mixing using a vortex mixer and the organic layer was counted for radioactivity with Topcount® (Packard Instrument Company, Inc., Meriden, CT, USA) using Microscint-0® (Packard). In the experiments of in vitro reversibility (Figs. 3 and 4), pre-incubation and incubation times were changed as described in the text.

In ex vivo MAO determination of the brain, C57BL/6 mice were orally administered the test compounds. The brains were removed 1 h later and frozen on dry ice and kept at –80°C until further assay. The brain tissue was homogenized with Polytron® in tenfold volume of cold 0.32 M sucrose and 10 mM sodium phosphate buffer (pH 7.4). Determination of MAO activity was carried out essentially by the methods described above. The brain homogenate was further diluted ninefold with 50 mM phosphate buffer, and the temperature of the diluted homogenate and tubes was stabilized for 10 min at 38°C in a water-bath incubator. Then \[^{14}C\]5-HT or \[^{14}C\]PEA was added and the homogenate was further incubated exactly 3 min or 2 min, respectively. The final concentration of the brain homogenate was 0.9% (w/v).

To evaluate simple dissociation of inhibitors from the enzyme, recovery of MAO activity of mitochondria was determined after repeated washing (Fig. 3). Mitochondria were pre-incubated with test compounds for 90 min at 38°C, and then unbound inhibitors were removed by repeated buffer change five times by centrifugation at 15,000×g and re-suspension in 50 mM phosphate buffer at 4°C. Concentrations of test compounds were adjusted to give about 75% inhibition, IC\textsubscript{50}, calculated according to the dose-response curve for IC\textsubscript{50} determination. Compounds and concentrations used for assay were 1 nM clorgyline, 2000 nM RS-8359, 1000 nM RS-1622, 30 nM RS-1636, 20 nM RS-1650 and 70 nM RS-1653 for MAO-A and 8 nM l-deprenyl, 200 nM lazabemide, 200 nM RS-1622, 20 nM RS-1636, 20 nM RS-1650 and 20 nM RS-1653 for MAO-B. Control mitochondria were treated using the same procedure without inhibitors to define 100% MAO activity. For the control of inhibition without washing, mitochondria were stood on ice during repeated centrifuge and re-suspension. Percent inhibition was separately calculated for samples with and without washing. To evaluate incubation-time dependency (Fig. 4), pre-incubation time was varied from 0 to 180 min and the MAO activity was determined as described above.
**Inhibition of reserpine-induced ptosis**

To evaluate MAO-A inhibition in vivo, the antagonistic effect on reserpine-induced ptosis was tested (13, 14). Reserpine at 2 mg/kg was given to C57BL/6 mice subcutaneously just after oral administration of the test compounds. Mice were placed on a stage 120 min after administration to score the ptosis as follows: 0, no ptosis; 1, closure of eyelid less than 1/2; 2, closure of eyelid more than 1/2; and 3, complete closure of the eyelid. The labels of the test solutions were “blinded” and the sequence of administration to each animal was randomized. The labels and sequence were decoded after the experiment, and the ptosis inhibition was calculated using the mean ptosis score of the vehicle-treated group as 100%. Six animals were used for each dose.

**Enhancement of PEA-induced locomotion**

To evaluate MAO-B inhibition in vivo, potentiation of PEA-induced locomotion was tested (15). Each C57BL/6 mouse was given one of the test compounds and allowed to habituate to a plastic box (17-cm W × 28-cm L × 17-cm H) on a locomotion count apparatus (AUTOMEX-II; Columbus Instruments, Columbus, OH, USA). Thirty minutes later, PEA at 25 mg/kg was given intraperitoneally (i.p.) after which the locomotion for 20 min was counted. As the double dose of PEA (50 mg/kg) induced the maximum effect of vigorous running and jumping response of about 2000 counts/20 min, the estimated dose of compound to induce 1000 counts/20 min was defined as the ED\textsubscript{50}. Five mice were used for each dose.

**RESULTS**

**Selectivity of MAO-A and -B inhibition**

The inhibition of the MAO-A and -B activity of mouse brain mitochondria was examined in vitro with \([^{14}\text{C}]\)-5-HT and \([^{14}\text{C}]\)-PEA as substrates, respectively. The deamination rate of the substrates was linear to incubation time during the 20-min incubation at 38°C with the 0.1 mg/ml-protein concentration of mitochondria used here. Table 1 shows the IC\textsubscript{50} values, the estimated concentrations at which the MAO inhibitors yield 50% MAO inhibition. The selectivity of known MAO inhibitors accorded well with prior reports (1, 2). \(l\)-Deprenyl and lazabemide selectively inhibited MAO-B. As indicated in Table 1, \(l\)-deprenyl required 383 times higher concentration for 50% inhibition of the \([^{14}\text{C}]\)-5-HT deamination than \([^{14}\text{C}]\)-PEA deamination. Clorgyline, moclobemide and RS-8359 selectively inhibited MAO-A. These results well accorded with known selectivity (1, 2), validating the highly selective enzyme assay in our procedures. Tranylcypromine inhibited both MAO-A and -B with similar concentrations.

Various selectivities of MAO-A and -B inhibition were obtained by changing the residues on the benzisoxazole ring (Table 1). Several MAO inhibitors, which have dual action to both MAO-A and MAO-B, were obtained as well as selective inhibitors. RS-1592 and RS-1530 selectively inhibited MAO-A while RS-1533 and RS-1519 were selective to MAO-B. Residues at the 7-position tended to increase MAO-A selectivity and halogen at the 5-position tended to increase MAO-B selectivity.

Oral administration of some of these MAO inhibitors inhibited brain MAO-A and -B (Table 2). These ex vivo results of brain tissue indicate some of the 3-(2-aminoethoxy)-1,2-benzisoxazole derivatives can be absorbed through the intestine and across the blood-brain barrier.

**Reversibility of MAO-A and -B inhibition**

In vivo MAO-A and -B inhibition was roughly estimated by two behavioral tests (Table 3 and Fig. 1). Reserpine-induced ptosis was antagonized by the MAO-A inhibitors clorgyline and RS-8359, but not by MAO-B selective inhibitor lazabemide. On the other hand, PEA-induced locomotion was potentiated by the MAO-B inhibitors \(l\)-deprenyl and lazabemide, but not by RS-8359, which is highly selective to MAO-A. To evaluate the reversibility, the effective dose given 1 day before was compared to the effective dose given just before the test. The irreversible inhibitors clorgyline, \(l\)-deprenyl and tranylcypromine gave similar ED\textsubscript{50} values even when administered 1 day before the assay, but the reversible inhibitors RS-8359 and lazabemide needed more than 10 times the dose. All of the benzisoxazole derivative compounds presented in Table 3 seem to have the characteristic of reversible inhibition to MAO-A. In MAO-B inhibition estimated by the PEA test, RS-1622, RS-1653 and RS-1655 were reversible. However, RS-1636 and RS-1650 maintained the effect one day after the administration, and the 1 day/0.5 h ratio of effective dose was not very different from that of tranylcypromine, an irreversible MAO inhibitor.

The time course of brain MAO inhibition after administration of some compounds was further tested by ex vivo study in mice (Fig. 2). Brain MAO-A and -B activity was quickly inhibited after oral administration of RS-1622 and both MAO-A and -B activity soon recovered. However, after administration of RS-1636, recovery of MAO-B was much slower than that of MAO-A. RS-1653 had similar differential reversibility, but its difference between MAO-A and -B inhibition was less significant than that of RS-1636. These results of differential recovery of MAO-A and -B correlated well with the in vivo reversibility indicated in Table 3.

**Mechanism of differential reversibility**

To evaluate simple dissociation of inhibitors from MAO, recovery of MAO activity was tested in vitro (Fig. 3). MAO
Differential Reversibility of MAO Inhibitor activity after repeated washing was determined at a cold temperature at which the enzyme activity is negligible. Mouse mitochondria were pre-treated with compounds for 90 min at 38°C, then the buffer was replaced five times by repeated spin and re-suspension at 4°C. The recovering rate was calculated using inhibition of each compound without washing as 100%. Inhibition of MAO-A by the competitive inhibitor RS-8359 was clearly removed (98.5%) and the inhibition of MAO-B by the mechanism-based inhibitor lazabemide was partially removed (33.5%). MAO activity was not recovered after treatment with the irreversible inhibitor clorgyline for MAO-A (9.2%) or l-deprenyl for MAO-B (4.4%). Inhibition of MAO-A by RS-1622 (36.6%) as well as lazabemide, but little MAO-B was recovered after incubation with RS-1636 (5.5%) and RS-1650 (9.8%). Some MAO-B recovery was observed after RS-1653 treatment (14.1%), but it was not very different from that of RS-1636.

To evaluate the association and dissociation profiles of the inhibitors to the MAO enzyme, incubation-time dependency was tested in vitro (Fig. 4). When the inhibi-

### Table 1. Selectivity of MAO inhibition in vitro

| Compounds     | Residues on benzoxazole | IC_{50} (nM) | Ratio 5-HT (MAO-A) / PEA (MAO-B) | Ratio 5-HT/PEA |
|---------------|-------------------------|-------------|---------------------------------|---------------|
| RS-1592       | H H C                   | 9.0         | 4,200                           | 0.002         |
| RS-1530       | H H C                   | 12.0        | 2,200                           | 0.005         |
| RS-1650       | CH_{3} H CH_{3}         | 3.3         | 13                              | 0.25          |
| RS-1636       | CH_{3} H CH_{3}         | 3.9         | 7.2                             | 0.54          |
| RS-1524       | H H C                   | 11.5        | 20.0                            | 0.58          |
| RS-1653       | F H C                   | 28.5        | 5.3                             | 5.4           |
| RS-1610       | CN H C H               | 120         | 6.8                             | 18            |
| RS-1622       | H H N                   | 195         | 13.5                            | 14            |
| RS-1655       | CF_{3} H N             | 510         | 9.8                             | 52            |
| RS-1627       | CF_{3} Br C H          | 470         | 0.44                            | 1,068         |
| RS-1533       | H Br C H               | 9,500       | 0.32                            | 29,688        |
| RS-1519       | H Cl C H               | 20,500      | 0.37                            | 55,405        |
| Clorgyline    |                         | 0.56        | 440                             | 0.0013        |
| Moclobemide   |                         | 41,000      | >100,000                        | <0.41         |
| RS-8359       |                         | 410         | 910,000                         | 0.0005        |
| Tranylcypromine|                        | 180         | 64                              | 2.8           |
| l-Deprenyl    |                         | 1,800       | 4.7                             | 383           |
| Lazabemide    |                         | >100,000    | 11                              | >9,000        |

Residues on 3-(2-aminoethoxy)-1,2-benzoxazole are shown in the table. IC_{50} values of MAO-A and -B were determined by in vitro MAO assay of mouse brain mitochondria using 5-HT or PEA as specific substrate, respectively.

### Table 2. ED_{50} of ex vivo MAO inhibition in C57BL/6 mice brain

| Compounds     | ED_{50} (mg/kg, p.o.) | Ratio 5-HT (MAO-A) / PEA (MAO-B) | Ratio 5-HT/PEA |
|---------------|-----------------------|---------------------------------|---------------|
| RS-1636       | 1.4                   | 1.5                             | 0.9           |
| RS-1524       | 1.6                   | 1.7                             | 0.9           |
| RS-1653       | 1.6                   | 1.3                             | 1.2           |
| RS-1594       | 2.0                   | 1.6                             | 1.3           |
| RS-1610       | 1.8                   | 0.63                            | 2.8           |
| RS-1622       | 27                    | 5.9                             | 4.6           |
| Clorgyline    | 4.1                   | >30                             | <0.14         |
| l-Deprenyl    | >10                   | 1.8                             | >5.6          |
| Lazabemide    | >1                    | 0.25                            | >4            |

Compounds were administered orally 1 h before decapitation. The ED_{50} of MAO inhibition was calculated from the dose-response curve.
K. Yoshimi et al.

Inhibition is irreversible, enzyme inhibition at the low concentration is expected to increase gradually with time (5, 6).

Table 3. Reversibility of MAO inhibitors in the in vivo behavioral tests of C57BL/6 mice

| Compounds  | Anti reserpine (MAO-A) | PEAs (MAO-B) |
|------------|------------------------|--------------|
|            | ED50 (mg/kg, p.o.)     | Ratio        | ED50 (mg/kg, p.o.) | Ratio        |
|            | 0 h        | 1 day       | 1 day/0 h | 0.5 h | 1 day | 1 day/0.5 h |
| RS-1653    | 7.4       | >=100       | >=13     | 0.52   | >10   | >19     |
| RS-1622    | 13        | >=300       | >=23     | 0.68   | >10   | >14     |
| RS-1636    | 1.5       | 113         | 75       | 0.55   | 2.6   | 4.7     |
| RS-1650    | 1.9       | 49          | 26       | 0.77   | 2.6   | 3.4     |
| RS-1655    | 10        | 100         | 10       | 0.48   | >10   | >21     |
| Clorgyline | 6.7       | 10          | 1.5      |        |       |         |
| Moclobemide| 1.4       | >100        | >71      |        |       |         |
| RS-8359    | 10        | 132         | 13       | >100   |       |         |
| Tranylcypromine | 2.4     | 4.2         | 1.7      | 0.66   | 1.4   | 2.1     |
| Deprenyl   |            |             |          | 2.2    | 2.8   | 1.3     |
| Lazabemide | >=10      |             |          | 0.11   | >10   | >=91    |

ED50 values at the beginning of assay or 24 h before assay in reserpine-induced ptosis and enhancement of PEA-induced locomotion tests are compared. Some examples are also shown in Fig. 1.

Fig. 1. Dose-response and reversibility of MAO inhibitors in the in vivo behavioral tests of C57BL/6 mice. Inhibition of reserpine-induced ptosis (panels A and B) and enhancement of PEA-induced locomotion (panels C and D). Compounds (A: RS-8359, B: RS-1622, C: lazabemide, D: RS-1636) were administered orally at the beginning of assay (open circles) or 24 h before the assay (closed squares). Values are means ± S.E.M. of 5 – 6 animals.
Differential Reversibility of MAO Inhibitor 179

by a competitive inhibitor RS-8359 had no time-dependent (Fig. 4B). Time-dependent increase of inhibition up to 30 min was observed for RS-1622 and RS-1636 (Fig. 4: C and D), but no increase was observed after. At the lower concentration (10^{-9} M) of RS-1636, MAO-A activity markedly recovered at 180 min.

MAO-B inhibition by l-deprenyl, an irreversible MAO-B inhibitor, clearly increased with time (Fig. 4E). MAO-B inhibition by RS-1622 (Fig. 4G) was free from time-dependent increase. Time-dependent increase of MAO-B inhibition by RS-1636 (Fig. 4H) was limited in the first 10 min, which was similar for the mechanism-based inhibitor lazabemide (Fig. 4F).

Fig. 2. Time course of ex vivo brain MAO inhibition of C57BL/6 mice by (A) RS-1622 (30 mg/kg, p.o.), (B) RS-1636 (3 mg/kg, p.o.) and (C) RS-1653 (10 mg/kg, p.o.). Percent inhibition of MAO-A (closed diamonds) and MAO-B (open circles) are shown. Values are means ± S.E.M. of 4–5 animals.

DISCUSSION

Differential reversibility of novel MAO inhibitors

Potent MAO inhibitors of various A and B selectivities were obtained among novel 3-(2-aminoethoxy)-1,2-benzisoxazole derivatives. Several compounds, such as RS-1622, RS-1636, RS-1650, RS-1653 and RS-1655, were dual inhibitors for both MAO-A and -B. They were short-acting inhibitors of MAO-A, but each compound had characteristic reversibility of MAO-B inhibition in the in vivo behavioral tests.

This differential reversibility was clearly confirmed by the time course of brain MAO activity (Fig. 2). MAO-A activity inhibited by RS-1636 was recovered soon, while MAO-B inhibition by the same compound lasted for a long period. RS-1653 had a differential time course similar to RS-1636. On the other hand, RS-1622 had a simple short-acting time course for both MAO-A and -B inhibition. In vitro, the MAO-A and -B activity was partially recovered after washing out RS-1622 (Fig. 3) and inhibition by RS-1622 did not depend on the incubation time (Fig. 4).

In general, pharmacological effects depend on the tissue concentration of the pharmacological compounds. Differential reversibility of MAO-A and -B inhibition by RS-1636 in the same brain subjects suggests either MAO-A or -B inhibition does not correlate with the time-course of concentration of free inhibitor in the brain tissue. MAO-A inhibition may depend on the concentration of free inhibitor, but it is not possible for the duration of MAO-B inhibition to correlate with the tissue concentration as well. Free l-deprenyl is known to disappear from the blood and brain within a few hours after administration, but its MAO-B inhibition lasts for several days because of its irreversible bond to MAO (8, 16, 17). This suggests RS-1636 and RS-1653 may bind to MAO-B tightly like irreversible inhibitors, but binds rather loosely to MAO-A.

Mechanisms of differential reversibility

Two possible mechanisms of MAO recovery, 1) simple dissociation and 2) enzymatic dissociation, were examined in vitro. Our results suggest that the differential reversibility observed in vivo and ex vivo is primarily a result of simple dissociation, and secondarily by enzymatic deamination by MAO activity. Moderate MAO-A recovery after repeated washing at 4°C (Fig. 3) explains the short-acting inhibition on MAO-A. RS-1622, RS-1636, RS-1650 and RS-1653 can dissociate from MAO-A at a considerable rate, so their MAO-A inhibition in vivo appears to depend on the concentration of free inhibitor in the tissue. However, while RS-1622 can be removed from MAO-B, RS-1636 and RS-1653 seem to bind to MAO-B tightly. The binding of RS-1636 to MAO-B seems a little tighter than that of RS-1653 (Fig. 3), and this may explain the longer duration
of MAO-B inhibition by RS-1636 shown in Table 3 and Fig. 2.

The MAO-A activity inhibited by 10 nM of RS-1636 recovered after extended incubation at 38°C in vitro (Fig. 4D). This indicates the decrease in the concentration of active inhibitors in the reaction solution, which suggests
decomposition of inhibitors by the enzymatic activity. As each of the 3-(2-aminoethoxy)-1,2-benzisoxazole derivatives has an amine residue, these inhibitors bound to MAO might be deaminated by the MAO themselves. While the binding property is tight-binding, MAO may release those inhibitors very slowly by enzymatic deamination.

**Potential usefulness of a dual inhibitor of MAO-A and -B**

In clinical use as antidepressants, MAO-A inhibitors should be reversible in order to avoid the potentiation of tyramine-induced hypertension, called the “cheese effect” (18, 19). To avoid the pressor effect of tyramine, a new generation of MAO inhibitors that are reversible MAO-A inhibitors (RIMA) have been developed (2, 18). On the other hand, the cheese effect of l-deprenyl, an irreversible MAO-B inhibitor, is very low, which makes it a safe anti-Parkinsonian drug (8). Long-acting MAO-B inhibition is preferable for the therapy of Parkinson’s disease, because high-doses of lazabemide are required to expand the duration of action due to its reversibility (20, 21).

Thus, the differential reversibility of RS-1636 and RS-1653 may open a new category of MAO inhibitors that act on both MAO-A and -B and have a tolerable tyramine pressor effect. The potential usefulness of a dual inhibitor to MAO-A and -B has been suggested in the literature (10, 22, 23). There are a few reports of strong anti-Parkinsonian effects of non-selective MAO inhibitors (24 – 26). As many Parkinson’s disease patients have moderate depression (27 – 29), the anti-depressant effect of MAO-A inhibition is desirable. Possible benefit of a reversible MAO-A inhibitor in Parkinson’s disease treatment has been suggested from clinical trials (30, 31). MAO-A and -B inhibition not only has an acute effect but also may save nigral neurons. Because neurons in the substantia nigra have MAO-A (32, 33), additional MAO-A inhibition may reduce radical production by dopamine metabolism in the cytoplasm of nigral neurons (10, 22). Fahn and Chouard (23) showed that tranylcypromine, an irreversible inhibitor of MAO-A and -B, can slow the progress of Parkinson’s disease, but extreme caution is needed to avoid the presser response by tyramine. A new dual MAO inhibitor that is reversible on MAO-A may make this kind of therapy widely practical.

**Reversibility optimization in drug development**

This profile of differential reversibility presents a new concept in drug design. IC\textsubscript{50} values in Table 1 indicate RS-1636 has weak MAO-A selectivity at short incubation time (20 min) in vitro. Only a half concentration of RS-1636 is needed to achieve 50% inhibition of MAO-A compared to MAO-B. However, interestingly, RS-1636 shows MAO-B selective inhibition 4 – 24 h after administration (Fig. 2B). In order to minimize adverse effects of therapeutic compounds acting on more than one target molecule (isoenzymes or receptor subtypes), it is essential to balance these actions. A profile of differential reversibility, in addition to IC\textsubscript{50} values, would give useful information for optimizing the balance.

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