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Chiral Resolution of the Enantiomers of the Slow-Onset Dopamine Reuptake Inhibitor CTDP-32476 and Their Activities

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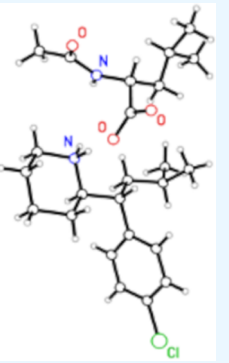
ABSTRACT: An improved synthesis was developed for CDTP-32476, a potent slow-onset dopamine reuptake blocker that may have utility as a treatment for cocaine abuse. The enantiomers of the compound were separated by fractional crystallization with *N*-acetylleucine enantiomers. An X-ray crystal structure was obtained of the RR enantiomer paired with *N*-acetyl-D-leucine. Chiral chromatography showed that the resolved enantiomers were pure with little contamination by the other enantiomer. The enantiomers were tested for their ability to block the reuptake of monoamines at their respective transporters and to stimulate locomotor activity in mice. Both enantiomers potently blocked the reuptake of dopamine and stimulated locomotor activity in mice. The RR enantiomer that corresponds to the active RR enantiomer of methylphenidate was slightly more potent at the dopamine reuptake site. The RR enantiomer also was found to be about twice as selective for the dopamine transporter relative to the norepinephrine transporter, which may have clinical implications. A method for designing slow-onset stimulants is proposed since there is increasing evidence that such activity is an important factor in stimulants that may have limited abuse potential.



INTRODUCTION

Cocaine is a widely abused stimulant drug that blocks the dopamine transporter (DAT) that returns dopamine (DA) to presynaptic neurons after it has been released into the synapse.[1] The DA remains in the synapse, the neuron continues to fire, and this produces a DA spike in brain reward regions that is associated with the euphoria produced by cocaine. This appears to be the source of the abuse potential of cocaine. When a cocaine dose wears off, DA levels tend to drop below baseline, which is associated with dysphoria, which may result in taking more cocaine.[2] Chronic abusers of cocaine also tend to have chronically reduced levels of DA, and this may cause the abuser to want more cocaine.[3] Currently, there is no medication available to help abusers wean themselves off cocaine. A potential difficulty in using a DAT-blocking stimulant as a medication for treating cocaine abuse is that it may be as abusable as cocaine itself.

Methylphenidate (Scheme 1) is in clinical use as a treatment for attention deficit hyperactivity disorder (ADHD). Its mode of action is believed to be the blockade of DA and norepinephrine (NE) transporters. We previously performed a conformational analysis of methylphenidate and developed a pharmacophore for its blockade of DA transporters through a superposition of the pharmacophore with a potent reuptake blocker from a different structural class.[4] The pharmacophore was successful in explaining the consistent decrease in the activity of analogues of *N*-methyl methylphenidate compared with other classes of DAT blockers where an *N*-methyl group is optimal.[5] That is, the extra *N*-methyl group in methylphenidate analogues preferentially occupies the position required for ammonium hydrogen. Crystal structures of analogues of the active threo isomer of methylphenidate all have a similar conformation, which we believe is the biologically active form, in which the phenyl ring is trans to the amine nitrogen.[6]

We previously synthesized and tested a series of methylphenidate analogues in which its ester group was replaced by a variety of alkyl groups.[7] Similar to methylphenidate, the compounds have activity at DAT and NET sites, but insignificant activity at serotonin transporter (SERT) sites. While 3,4-dichloro groups on the phenyl ring produced the most potent compounds, these were less selective for DAT relative to NET sites and a 4-chloro group produced the most selective compounds for DAT. All compounds in this series for which we obtained crystal structures had a similar conformation to the proposed biological form of methylphenidate in which the phenyl ring is trans to the ammonium nitrogen.[8] There also appeared to be a limitation for the size of the alkyl group with larger groups having reduced activity. A

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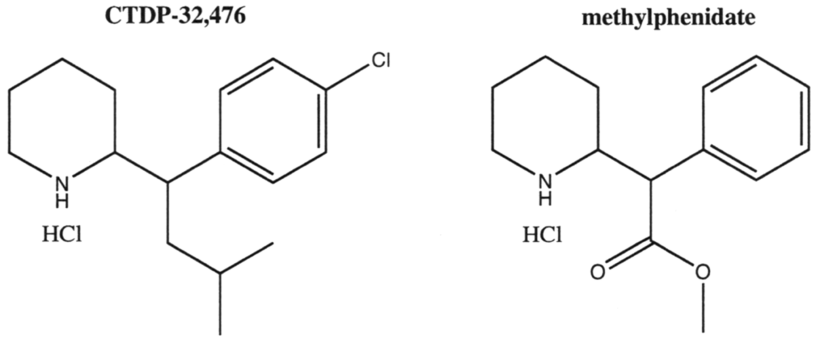
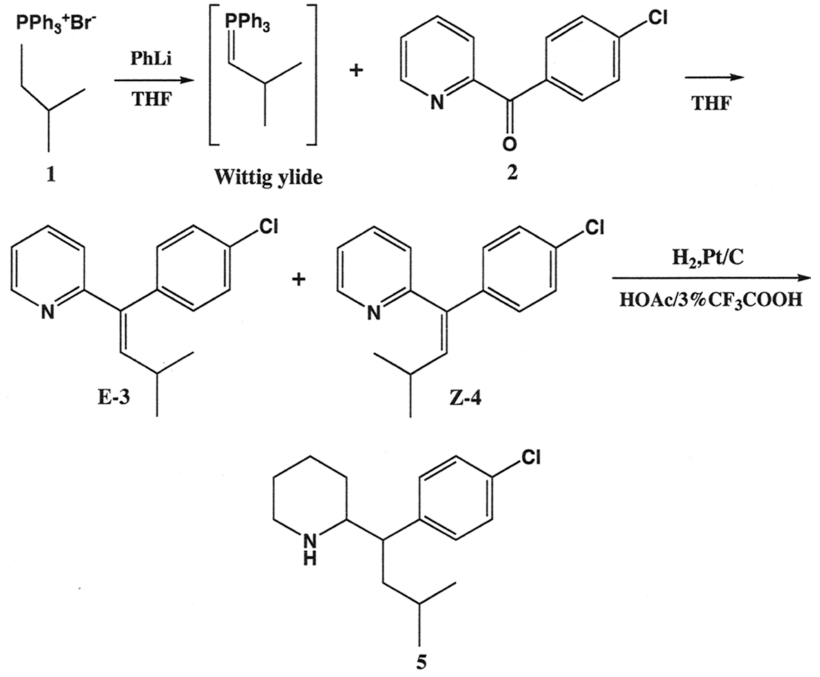
Scheme 1



Scheme 2



similar result was found for methylphenidate analogues where those with larger ester groups lost potency.[9]

One compound, CDTP-32476 (Scheme 1, compound 10f in Table 1),[7] was chosen for further characterization and has properties that may be useful in treating cocaine abusers. It is a potent blocker of DA reuptake with an affinity of 8.6 ± 2.9 nM and is moderately selective (14-fold) versus NE reuptake blockade with little effect on the SERT. Injected into rodents, it has a slow onset of at least 20 min and then slowly raises brain DA levels. This is important since fast stimulant onsets are associated with increased abusability, which is the case for cocaine which has a very rapid onset. Another desirable feature of CTDP-32476 is its duration of action which appears to be about twice that of methylphenidate and considerably longer than cocaine itself.

Because of its slow onset of action, the compound is not self-administered by naïve rats.[10] However, rats that have learned to consistently self-administer cocaine do self-administer the compound in experiments in which it has been substituted for cocaine, but at lower levels than cocaine itself (partial behavioral extinction).[10] Recently, we attempted to determine whether the partial behavioral extinction would continue to full extinction if the animals were given a longer period of extinction. We found that, after about 3 weeks, complete extinction did ensue with the animals self-administrating the compound at the same level as vehicle or saline.[11] This suggests that the slow onset property of the compound may indicate that it has little or no abuse potential.

CTDP-32476 and methylphenidate both have two asymmetric carbon atoms resulting in four possible isomeric structures, threo and erythro diastereomers, each of which consists of a pair of enantiomers. For methylphenidate, the active diastereomer is threo RR/SS and the active enantiomer is RR.[12−14] For 32 476, the active diastereomer is again RR/SS.[7]

RESULTS AND DISCUSSION

Synthesis. In prior work,[7] racemic CTDP-32476 was synthesized by the addition of isobutyl Grignard to 4-chlorophenyl 2-pyridyl ketone followed by the reduction of the pyridine ring, dehydration of the 3° alcohol, and catalytic hydrogenation of the piperidine-alkene. The procedure suffered from byproduct formation in the Grignard addition and irreproducibility in the dehydration step.

A shorter, more reproducible synthesis for the free base of 32 476 is shown in Scheme 2. The Wittig ylide, isobutyl-

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triphenylphosphonium ylide, was produced by adding phenyl lithium to isobutyltriphenylphosphonium bromide **1**. This interacted with 4-chlorophenyl 2-pyridyl ketone **2** (previously synthesized)[7] to produce the isomeric pyridine-olefins **3** and **4**. Catalytic hydrogenation of **3** and **4** gave a mixture of the diastereomers RS/SR and RR/SS of **5**, which were separated upon silica gel chromatography. Resolution of the RR and SS enantiomers was obtained by interacting racemic **5** with the chiral acids *N*-acetyl-L- and -D-leucine. After three recrystallizations, the salts were converted to free bases by aqueous KOH followed by extraction with dichloromethane to give resolved RR- and SS-**5**.

Chiral Chromatography. As can be seen from Table 1, the two enantiomers are well resolved with the chiral column and are >99% pure. Chromatograms of the racemate and its enantiomers are shown in Figure 1. We wanted to determine the sensitivity of the method for the detection of a small amount of enantiomeric impurity. As can be seen from Table 2, the method can detect a 0.5% spike of one enantiomer. That suggests that the chiral resolution achieved samples of the two enantiomers with little contamination of one enantiomer by the other. This is important in this case because both pure enantiomers have similar activities (see below).

Table 1. Retention Time, Tailing Factor (Tf), Resolution, and Peak Area of CDTP-32476

peak 1, min	peak 2, min	peak 1 Tf	peak 2 Tf	resolution	peak 1 area %	peak 2 area %
21.24	23.05	0.88	0.91	1.72	49.8	50.2

The two enantiomers were labeled ATDP-34209 and ATDP-34210. Their absolute configurations were determined by X-ray crystallography (see below), and ATDP-34210 is the RR enantiomer corresponding to the active RR enantiomer of methylphenidate.

Single-Crystal X-ray Diffraction Analysis. The crystal structure of the RR enantiomer of 32 476 bound to *N*-acetyl-D-leucine is shown in Figure 2. As we have consistently found in crystal structures of compounds in this series, the phenyl ring is trans to the amine nitrogen.

Table 2. Small Isomer Detection Accuracy

nominal level	0.5% isomer 2 in isomer 1	1% isomer 2 in isomer 1	0.5% isomer 1 in isomer 2	1% isomer 1 in isomer 2
		accurate spiking %		
% of isomer 1	99.57	99.14	0.57	1.14
% of isomer 2	0.43	0.86	99.43	98.86
		detected area %		
% of isomer 1	99.23	99.18	0.76	1.13
% of isomer 2	0.77	0.82	99.24	98.87

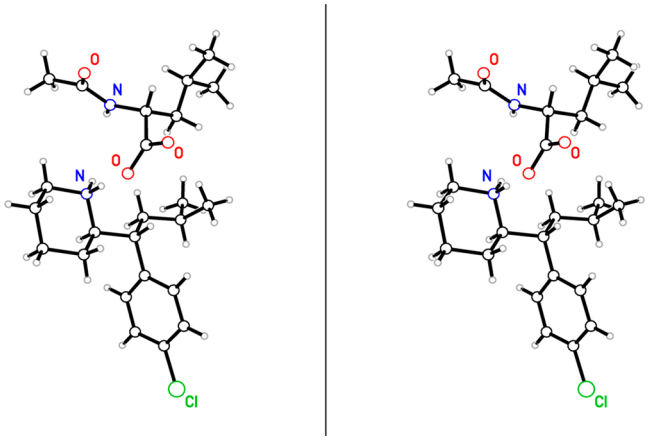

Figure 2. Stereo image of the crystal structure of RR enantiomer with *N*-acetyl-D-leucine.

Biogenic Amine Transporter Assays. The enantiomers and cocaine were tested in binding to and reuptake assays utilizing recombinant human DA, NE, and 5HT transporters stably expressed in human embryonic kidney 293 cells (HEK-

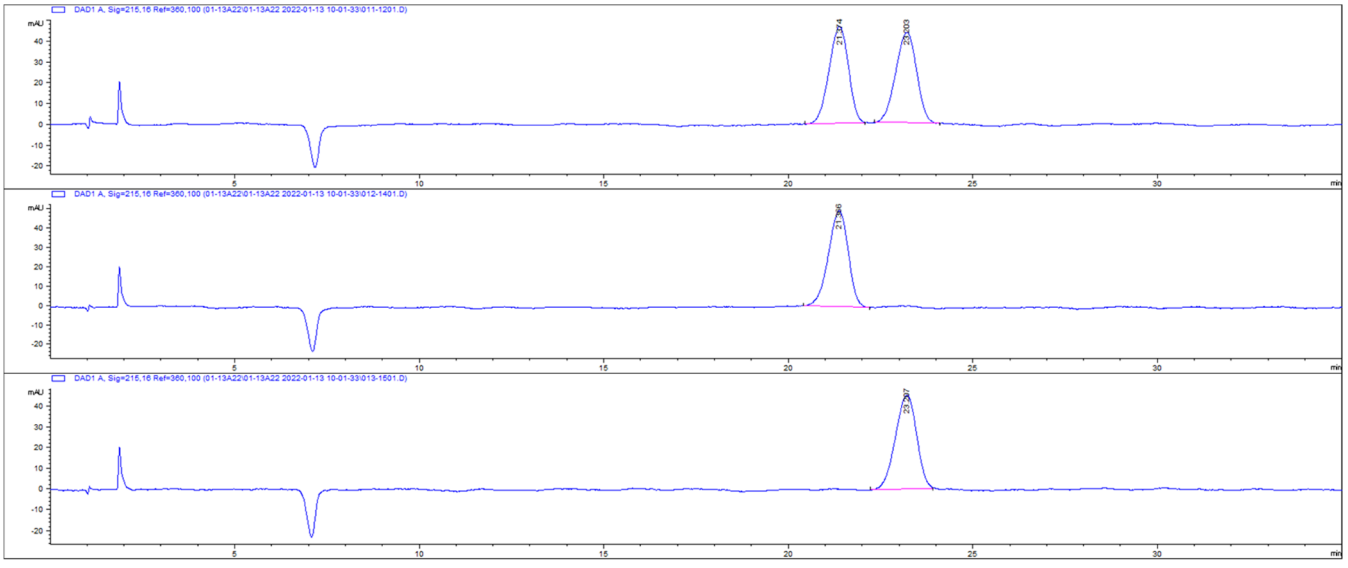

Figure 1. Chromatograms of racemate and its two enantiomers. Top: CTDP-32476, 0.5mg/ml in MeOH; middle: ATDP-34209, 0.25 mg/mL in MeOH; bottom: ATDP-34210, 0.25 mg/mL in MeOH.

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Table 3. Biogenic Amine Transporter Assays

	CDTP-32 476[7]	ATDP-34 209	ATDP-34 210	cocaine	methylphenidate[7]
HEK-hDAT cells					
[^{125}I] RTI-55 binding Ki (nM)	16 ± 4	13.5 ± 3.5	6.0 ± 2.0	770 ± 160	110 ± 9
[^{3}H] DA uptake IC_{50} (nM)	8.6 ± 2.9	13.0 ± 2.6	9.8 ± 2.3	276 ± 42	79 ± 16
HEK-hSERT cells					
[^{125}I] RTI-55 binding Ki (nM)	5900 ± 900	1830 ± 270	3320 ± 450	376 ± 39	65 000 ± 4000
[^{3}H] 5HT uptake IC_{50} (nM)	490 ± 80	4590 ± 540	>10 000	343 ± 56	5100 ± 7000
HEK-hNET cells					
[^{125}I] RTI-55 binding Ki (nM)	840 ± 130	640 ± 110	416 ± 74	1850 ± 200	660 ± 50
[^{3}H] NE uptake IC_{50} (nM)	120 ± 40	123 ± 16	210 ± 24	410 ± 100	61 ± 14
DA uptake/NE Uptake	14	9.5	21	1.5	0.77

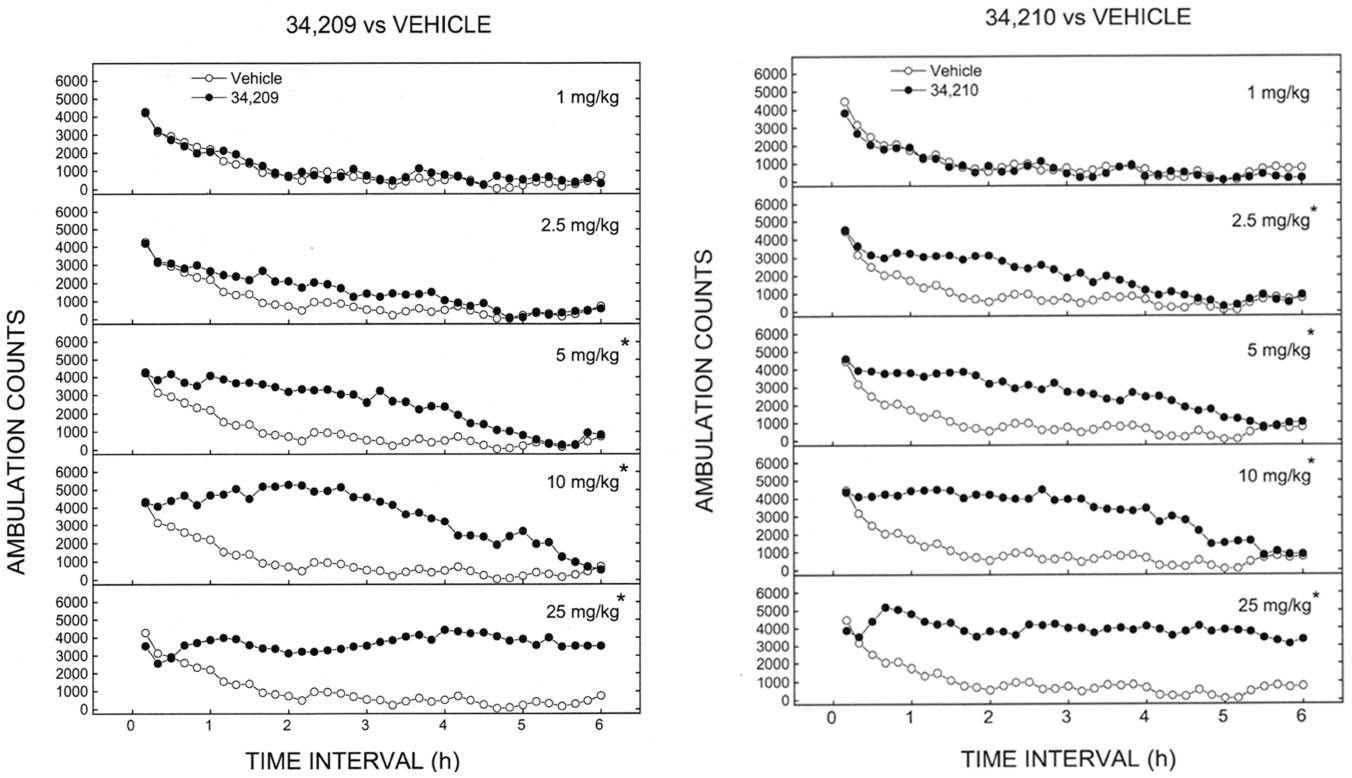

Figure 3. Effect of 34 209 and 34 210 on horizontal activity counts as a function of dose during a 6 h session.

hDAT, -hNET, and -hSERT). The binding studies measured the potency of the enantiomers in combination experiments with [^{125}]RTI-55, while the reuptake assays measured their potency in inhibiting the uptake of the tritiated monoamines. Results are shown in Table 3. As has been found repeatedly, cocaine is approximately equipotent at all three monoamine reuptake sites. Previous results for methylphenidate[7] are included that show that methylphenidate has little effect on the 5HT reuptake site and are approximately equipotent for the DA and NE sites.

With respect to the ability of the two enantiomers to block the reuptake of DA to the hDAT, 34 210 was more potent than 34 209 with an IC_{50} of 9.8 ± 2.3 nM compared to an IC_{50} of 13.0 ± 2.6 nM for the latter. For the reuptake of NE to the hNET, the IC_{50} was 210 ± 24 nM for 34 210 and 123 ± 16 nM for 34 209. This makes 34 210 more selective for blocking DA reuptake relative to blocking NE reuptake which may make it more desirable as a medication. As expected, both enantiomers have little effect at the hSERT.

There is a discrepancy in the IC_{50} for the racemate in the hSERT assay relative to the IC_{50}'s of the enantiomers with the former being much more potent than would be expected based on the latter. We believe that the IC_{50} of the racemate is in error. Evidence that the IC_{50} of the racemate should have been weaker can be seen from the binding K_i, which makes it a very weak compound at the site. Also, the IC_{50} for methylphenidate, which was the prototype for our compound series, is quite weak at the hSERT site. It should be noted, however, the anomalous value still makes the racemate quite weak at the hSERT site since it is 57 times weaker than the IC_{50} at the hDAT site. Thus, this apparent error is of no consequence.

As has been found repeatedly, cocaine is approximately equipotent on all three monoamine reuptake sites (Table 3). Both enantiomers of 32 476 are considerably more potent than cocaine at both the hDAT and hNET. The SS enantiomer of 32 476 had 77% of the potency of the RR enantiomer in blocking the reuptake of DA, which makes it a still quite potent compound. With respect to NE reuptake blockade, the SS enantiomer is about 1.7 times the potency of the RR

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enantiomer. It is surprising that both enantiomers are potent in these assays. This differs from methylphenidate where only the RR enantiomer has activity.[12] This can be understood by the substitution of a nonpolar alkyl group for the polar ester group in methylphenidate. That is, a polar group in the wrong position in binding to a receptor site is more detrimental to binding, whereas nonpolar alkyl groups in the wrong position appear to be more tolerated. Other examples of compounds in this series with a favorable group producing potent compounds despite unfavorable structures are those with 3,4-dichlorophenyl substitutions. Despite being an unfavorable RS/SR diastereomer, the compound with this substitution and the isobutyl sidechain has an IC_{50} of 13.3 ± 3 nM.[7] A recent example of this is a cathinone analogue with a 3,4-dichlorophenyl group is a more potent DA reuptake inhibitor, whereas other phenyl substitutions are considerably weaker.[15]

While we report both the binding affinities and reuptake blockade potencies, we believe that the latter are more relevant since they are obtained from a physiological measurement.

Mouse Locomotor Assay. The locomotor results for 34 209 and 34 210 are shown in Figure 3. As found previously for the racemate, both enantiomers had slow onsets of locomotor stimulation. At the highest dose, the stimulatory effect lasts more than the 6-hour test period. Comparing the locomotor assay results of the two enantiomers with that of methylphenidate (see ref 7), it appears that they last about twice as long as the latter at what appear to be approximately comparable 10 mg/kg doses, which would be a desirable feature that would require fewer doses.

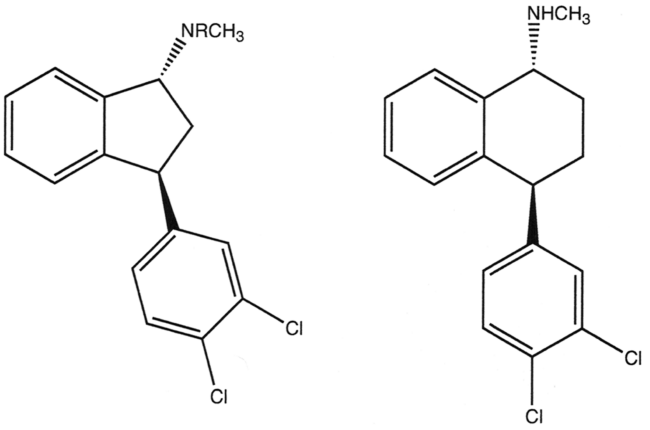
Based on a linear regression against $\log_{10}$ doses of the compounds, the ED_{50}'s of the racemate and enantiomers in the locomotor assay are shown in Table 4. Paralleling the results of the biogenic amine assays, 34 210, whose structure matches the active enantiomer of methylphenidate, is the more potent of the two.

Table 4. ED_{50} in Mouse Locomotor Assays

	ED_{50} (mg/kg)
32 476[7]	3.3
34 209	3.3
34 210	3.1

Creating DA Reuptake Blockers with Little or No Abuse Potential. The source of the characteristic slow onset of our compounds needs an explanation. Comparing our compounds with fast-onset methylphenidate, the most obvious change is the replacement of the polar ester group by a hydrophobic group, which appears to slow the delivery of the compound into the brain. When 32 476 is administered intracranially, it has a fast onset of activity, suggesting that delivery into the brain is responsible for its slow onset when administered peripherally.[10] This seems consistent with other series that we have studied such as trans-3-phenyl-1-indanamines (R = CH_3; Scheme 3), which also have an onset delay of at least 20 min.[16] Other researchers have also indicated that a compound in this class (R = H; Scheme 3), known as LU-19-005 or indatraline, has a slow onset.[17,18] A second series with a slow onset is the trans-1-amino-4-phenyltetralines (Scheme 3).[19] The consistent difference between these slow- and fast-onset compounds like cocaine and methylphenidate is the absence of a polar ester group in the former, and this suggests a strategy for the design of slow-onset stimulants with little or no abuse potential.

Scheme 3



3-phenyl-1-indanamines 1-amino-4-phenyltetralins

It is important to note that cocaine-like compounds act as antagonists since they are blocking the reuptake of synaptic DA into presynaptic neurons. That may be the reason that both enantiomers of 32 476 can have similar pharmacological activities. That is, to be an antagonist, all that needs to occur is that the compound binds strongly to the target receptor. This differs from the situation for agonists where the compound needs to activate the receptor mechanism. For example, for some DA compounds, one enantiomer is an agonist while the other enantiomer is an antagonist.[20] The agonists have the correct configuration to activate the receptor while the structurally similar antagonists bind to the receptor, perhaps to the same site.

Based on the results of an experiment where rats learned to self-administer cocaine and were then switched to 32 476 which reduced their self-administrations to the same level as saline or vehicle within about three weeks,[11] the crucial factor for a compound with limited abuse potential is a slow onset. With a compound like this, a potential abuser never gets the synaptic DA spike associated with euphoria. Based on the structure of 32 476 and other compounds which have this property, the key appears to be making the compound relatively nonpolar, which slows its ability to penetrate the brain. As indicated in the Introduction section, a compound like this may be clinically useful in slowly raising synaptic DA to ameliorate the deficits associated with chronic cocaine abuse. It is also important for the compound to have a longer half-life to reduce the number of times it must be taken, but not too long that might interfere with sleep at the end of the day. Based on their effects in the mouse locomotor assay, 32 476 appears to last approximately twice as long as methylphenidate with equivalent doses[7] and the latter requires two doses/day when it is not used in a special slow-release formulation. That should allow 32 476 to wear off by the end of the day.

There also appear to be certain substitutions such as 3,4-dichlorophenyl that enhance potency so that a variety of unexpected structures become sufficiently potent to become potentially useful as medications. For example, even the "wrong" RS/SR diastereomer with this substitution can have low nanomolar potencies as outlined above. Another example of this is a cathinone analogue in which the ester group is

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replaced by a carbonyl group.[15] This opens up the possibility of producing potent DAT reuptake blockers with structures that would normally not be considered.

Materials and Methods. Melting points were measured taken in open glass capillaries in an SRS DigiMelt apparatus and are uncorrected. NMR spectra were recorded on a Varian 300 MHz spectrophotometer. Reactions were monitored by thin-layer chromatography on silica gel with hexane/ethyl acetate mixtures containing 1% added diethylamine. Necessary chemicals and solvents were available from Aldrich or Lancaster.

Synthesis. The Wittig ylide, isobutyltriphenylphosphonium ylide, was produced by adding phenyl lithium (13.9 mL, 1.9 M) dropwise at room temperature over 12 min to a stirring slurry of isobutyltriphenylphosphonium bromide 1 (10.1 g, 25.3 mmol) in THF (100 mL) under argon. The initial yellow color darkened to pomegranate red, and the solution was chilled to −55°. A solution of 4-chlorophenyl 2-pyridyl ketone **2** (5.00 g, 23.0 mmol)[7] in THF (20 mL) under argon was added via a syringe while the temperature was maintained between −55 and −50°. The cold bath was removed, and the reaction warmed slowly to room temperature and stirred for an hour. The tan solution was quenched with 75 mL of sat. aq. NH_4Cl. The organic layer was decanted to a separatory funnel and shaken twice with 75 mL portions of NH_4Cl. Solids in the lower layer were drained through the stopcock. The organic layer was shaken with 50 mL of brine, and THF was removed in vacuo to give a solid residue. The solids were transferred with 130 mL of diisopropyl ether to an Erlenmeyer flask, digested at reflux, cooled, and filtered to give 4.44 g of triphenylphosphine oxide. The filtrate was stored at −13° for two days as further triphenylphosphine oxide precipitated on the walls of the flask. The solution was decanted and concentrated to a light brown oil (5.82 g, 95%) of crude pyridine−alkene mixture (40% E, 60% Z diastereomers by NMR) containing traces of starting ketone and triphenylphosphine oxide.

The crude alkenes **E-3** and **Z-4** were purified by Combiflash chromatography on silica gel using a gradient of hexane/EtOAc (90:10 to 70:30). The first fraction crystallized to give pure **E-3**, mp 57-8 °C. ^{1}H NMR ($CDCl_3$) δ 8.58 (1 H, ddd, J = 1.2, 2.4, 4.1 Hz), 7.50 (td, J = 1.8, 7.6 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.16 (d, J = 8.2 Hz, 2H), 7.09 (ddd, J = 1.2, 3.7, 5.9 Hz, 1H), 6.86 (br. d, J = 8.2 Hz, 1H), 6.72 (d, J = 10.0 Hz, 1H), 2.40 (1H, d of septets, J = 10, 7 Hz, 1H), 1.02 (d, J = 7 Hz, 6H). ^{13}C NMR ($CDCl_3$) 158.2, 149.2, 141.0, 137.6, 137.3, 136.3, 133.0, 131.2 (2C), 128.6 (2C), 121.9, 121.6, 28.7, 22.9 (2C).

Z-4 was eluted as an oil. ^{1}H NMR ($CDCl_3$) δ 8.68 (m,1H), 7.71 (td, J = 2.3, 8.7 Hz, 1H), 7.22-2.27 (m, 2H), 7.23 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H), 5.97 (d, J = 10 Hz, 1H), 2.48 (d of septets, J = 10.0, 7.0 Hz, 1H), 1.04 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$) 158.8, 149.7, 140.0, 139.6, 137.8, 136.2, 132.7, 128.41 (2C), 128.27 (2C), 124.8, 121.9, 28.7, 23.0, (2C).

Catalytic hydrogenation of the initial mixture of **3**/**4** over 20% Pt/C in acetic acid/3% trifluoroacetic acid, gave a mixture of diastereomers. Isomer **5** (RS/SR, 63%) eluted before RR/SS-**5** (37%) upon silica gel chromatography.[7] Catalytic hydrogenation of the separated diastereomers **E-3** and **Z-4** each gave about the same ∼60:40 ratio of diastereomers.

Resolution of the SS Enantiomer. *N*-Acetyl-L-leucine (586 mg, 3.38 mmol) was dissolved in 70 mL of boiling EtOAc and allowed to cool. Chromatography fractions containing 0.906 g (3.41 mmol) of the free base of RR/SS-**5** were dissolved in 30 mL of warm ethyl acetate, and the solutions were mixed. Overnight, tufts formed in the flask giving 1.193 g of white micro-needles of salt [L, SS], mp 171−173°. This solid was dissolved in 30 mL of EtOAc, seeded, and overnight precipitated 0.552 g of matted needles, mp 177−179°. The procedure was repeated with two other chromatography fractions of 943 mg of RR/SS-**5** containing traces of triphenylphosphine oxide, and with 1.211 g of racemic RR/SS-**5** containing a trace of the RS/SR diastereomer. Each salt was recrystallized once from EtOAc. The combined crystals from the three runs (2.178 g) were dissolved in 110 mL of boiling acetonitrile and allowed to crystallize slowly. After filtration, washing with 30 mL of acetonitrile, and air-drying, 1.813 g (3.36 mmol) of needle crystals of [L, SS] salt, mp 180−180.5° (sharp) was obtained.

Resolution of RR Enantiomer. The combined parent liquors from the above crops were concentrated to dryness, dissolved in 50 mL of DCM, transferred to a separatory funnel containing 25 mL of 4 M KOH, and shaken four times with DCM. The combined DCM extract was dried (Na_2CO_3), filtered, and concentrated to give 1.15 g of crude **5** enriched in the RR-diastereomer. *N*-Acetyl-D-leucine (0.747 g, 4.32 mmol) was dissolved in 75 mL of boiling EtOAc, and a solution of enriched **5** (1.15 g, 4.32 mmol) in 25 mL of EtOAc was added. After 17 h at room temperature, the crystalline mass was filtered to give, after air-drying, 1.290 g of the enantiomeric salt [D, RR], mp 178−179.4°. This sample was combined with another sample (total weight 1.859 g), dissolved in 110 mL of boiling EtOAc, and boiled down to 90 mL. On cooling, the flask filled with crystals. After filtration and drying, 1.673 g (3.10 mmol) of the D, RR salt, mp 179.5−180.7°, was obtained. The sample was dissolved in 80 mL of acetonitrile and allowed to cool. Cottony needles formed spontaneously and were collected to give 1.589 g (2.95 mmol) mp (DigiMelt) 180.6−182°.

RR-**5** free base was obtained from a slurry of 0.82 g of the [D, RR]-salt in 20 mL of 2*M* KOH by extraction with a 25 mL portion of DCM followed by 3 × 10 mL of the same solvent. The combined organic layers were concentrated to an oil. ^{1}H NMR ($CDCl_3$) δ 7.25 (d, J = 8 Hz, 2H), 7.07 (d, J = 8 Hz, 2H), 3.04 (d br., J = 11 Hz 1H), 2.45−2.65 (m, 3H), 1.44−1.74 (m, 5H), 1.17−1.38 (m, 4H), 0.95 (qt, 1H), 0.82 (d, J = 7 Hz, 3H), 0.79 (d, J = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$) 141.3, 131.7, 129.8 (2C), 128.3 (2C), 61.9, 49.2, 47.4, 40.6, 30.7, 26.6, 25.2, 24.9, 24.1, 21.0. ATDP-34210 was prepared by dissolving a portion of RR-**5** in 95% EtOH and adding drops of conc. HCl. The liquids were removed under reduced pressure. The dry, solid residue gave cubic crystals on recrystallization from hot ACN. ^{1}H NMR ($CDCl_3$) δ 9.41 (broad s, NH_2, 2H), 7.31 (d, J = 8.9 Hz, 2H), 7.14 (d, J = 8.9 Hz, 2H), 3.70 (d br., J = 12.3 Hz 1H), 3.22 (ddd, J = 3.5, 8.8, 15.2 Hz, 1H), 2.82−3.03 (m, 2H), 1.86−2.04 (m, 2H), 1.68−1.75 (m, 3H), 1.45−1.55 (m, 2H), 1.14−1.40 (m, 2H), 0.86 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) 138.4, 133.0, 129.8 (2C), 129.1 (2C), 61.9, 46.1, 45.7, 41.0, 27.5, 25.3, 24.0, 22.5, 22.1, 20.9.

Chiral HPLC. Optimal conditions for the separation of the enantiomers on a chiral column were developed as follows: Column: Astec Chirobiotic T, 5 μm stationary phase with a dimension of 150 mm × 4.6 mm, 5 μm; detection: UV at 215

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nm; flow rate: 1.0 mL/min; injection size: 5 μL; isocratic elution: methanol, 70%, acetonitrile, 30%, triethylamine, .01%.

Single-Crystal X-ray Diffraction Analysis. A clear colorless needle crystal of dimensions 0.455 mm × 0.050 mm × 0.030 mm was mounted on a MiteGen MicroMesh using a small amount of Cargille Immersion Oil. Data were collected on a Bruker three-circle platform diffractometer equipped with a PHOTON II CPAD detector. The crystals were irradiated using a 1 μs microfocus Cu Kα source (λ = 1.54178) with Montel optics. Data were collected at room temperature (20 °C).

After data collection, the unit cell was initially refined using *APEX3* [v2015.5−2].[21] Data reduction was performed using *SAINT* [v8.34A][22] and *XPREP* [v2014/2].[23] Corrections were applied for Lorentz, polarization, and absorption effects using *SADABS* [v2014/2].[24] The structure was solved and refined with the aid of the program SHELXL-2014/7.[25] The full-matrix least-squares refinement on F^2 included atomic coordinates and anisotropic thermal parameters for all non-H atoms. Hydrogen atoms were located from the difference electron density maps and added using a riding model.

Biogenic Amine Transporter Assays. The compounds were tested for their effects on radioligand ([^{125}I]RTI-55) binding to and [^{3}H]DA uptake by HEK cells expressing cDNA for the human DAT (HEK-hDAT cells), their effects on radioligand ([^{125}I]RTI-55) binding to and [^{3}[H]-SERT uptake by HEK cells expressing cDNA for the human SERT (HEK-hSERT cells), and their effects on radioligand ([^{125I}]RTI-55) binding and [^{3}H]NE uptake by HEK cells expressing cDNA for the human NET (HEK-hNET cells) as previously described.[26]

Mouse Locomotor Assay. Male, Swiss−Webster mice were purchased from Envigo (Indianapolis, Indiana) at approximately 2 months of age and allowed to acclimatize for about 2 weeks prior to behavioral testing. Mice were group-housed (n = 4/cage), allowed free access to food, maintained on a 12:12 light/dark cycle (lights on at 7:00 AM), and had access to water. All housing and procedures were approved by the University of North Texas Health Science Center Institutional Animal Care and Use Committee and were in agreement with the guidelines set for the care and use of laboratory animals.

A time course/dose response study of cocaine-induced locomotor stimulation was conducted. The study used 32 Digiscan locomotor activity testing chambers (40.5 cm × 40.5 cm × 30.5 cm), each housed within a sound-attenuating chamber that provided dim illumination. A panel of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. Separate groups of 8 non-habituated male Swiss−Webster mice (Hsd:ND4, aged 2−3 mo) were injected intraperitoneally with either vehicle (0.9% saline), or one of the two enantiomers (1, 2.5, 5, 10, and 25 mg/kg) in a volume of 10 mg/kg, immediately prior to locomotor activity testing. Horizontal activity (interruption of photocell beams) was measured for 6 h within 10 min periods. Testing was conducted with one mouse per activity chamber.

CONCLUSIONS

An improved synthesis of the DAT reuptake blocker CDTP-32476 was developed utilizing a Wittig reaction. The compound's enantiomers were separated by fractional crystallization of salts with enantiomers of *N*-acetylleucine, and their absolute configuration was determined by X-ray crystallography. Chiral chromatography demonstrated that there was little contamination by the other enantiomer. Unexpectedly, both enantiomers were potent blockers of DA reuptake, stimulated mouse locomotor activity with similar potencies, and had slow onsets. The RR enantiomer, ATDP-34210, which corresponds to the active RR enantiomer of methylphenidate, was more selective in blocking DAT than NET. The surprisingly potent activity of the less active ADTP-34209 suggests that a nonpolar group in the same position as the polar ester group in methylphenidate is more tolerated in binding to the uptake receptor. The factor that makes our compound as well as others that have been identified as having slow onsets compared with cocaine and methylphenidate is the lack of polar groups in the former. Since slow-onset DAT reuptake blocker CTDP-32 476 may have little if any abuse potential, this suggests that a method to develop such future compounds may be achieved with molecules that are more nonpolar. These could be used as possible novel medications for treating stimulant abuse. Stimulants with little or no abuse potential may also be useful for other clinical applications.

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M.F. arranged for the testing of the enantiomers and wrote most of the manuscript. R.T. did the initial chemical work on enantiomeric resolution. Z.J.P. and D.J.R. did laboratory work under the supervision of C.J.K. G.H.I. obtained the crystal structure of one enantiomer bound to its chiral acid. E.L.G. participated in the writing of the manuscript. C.J.K. supervised the synthetic work and enantiomeric resolution.

Notes

The authors declare no competing financial interest.

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