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Short communication

# Synthesis and biological evaluation of 6/7-*exo*-methyl-3β-(4-iodo)phenyltropane-2β-carboxylic acid methyl esters

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**Abstract**

6β/7β-Methyl-2-methoxycarbonyltropinones (**3a**, **3b**) were synthesized and used as starting materials in the synthesis of 6β/7β-methyl-2β-methoxycarbonyl-3β-phenyltropanes (**6a**, **6b**), 6β/7β-methyl-2β-methoxycarbonyl-3β-(4-iodo)phenyltropanes (**7a**, **7b**) and 6β-methyl-2β-methoxycarbonyl-3β-(4-iodo)phenylnortropane (**8**). The effect of 6/7-groups was evaluated by in vitro receptor binding to dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters. Introduction of a methyl group at the 6- or 7-position diminished the overall affinity for the transporters, though mostly to NET. In vivo locomotor tests were performed in mice for compounds **7a** and **8**. Compound **8** had no apparent effect on locomotor activity. Compound **7a** increased locomotion in a wide dose range, but was much less potent than a reference compound, 2β-carbomethoxy-3β-(4-iodo)phenyl-tropane (β-CIT).


*Keywords:* Phenyltropanes; Synthesis; In vitro-binding; Locomotor studies

## 1. Introduction

Cocaine binds to the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters in the brain. Phenyltropanes are cocaine analogues, which have been evaluated in programmes seeking treatments for cocaine addiction and overdose [1]. Radiolabeled DAT and SERT selective phenyltropanes have also been developed for brain imaging with single photon emission tomography (SPET) and positron emission tomography (PET) [2].

Since the 1990s, when it was discovered that binding to the monoamine transporters can be influenced by modifications on the tropane skeleton, several hundreds of phenyltropanes with varying selectivity to DAT, SERT or NET have been prepared by modifying substituents on the N-, C2- and C3-positions of the bicyclic tropane ring [1]. Analogues with substituents at the C6/C7-bridge have also been prepared and studies with enantiopure C6/C7-hydroxylated phenyltropanes have shown increased DAT selectivity with similar potency to their unsubstituted counterparts [3,4]. However, substitution especially with bulky substituents has been found to reduce affinity to DAT and particularly to SERT [5–9].

In our study, we explored the influence of C6/C7-methyl group on binding to all three monoamine transporters, DAT, SERT and NET, by comparing it with the widely studied counterpart, 2β-carbomethoxy-3β-(4-iodo)phenyltropane (β-CIT), [10,11] in order to find new tools in developing more selective radioligands for transporter imaging with SPET/PET.

There are two main strategies for synthesis of the 6/7-substituted phenyltropanes; a strategy exploiting pyridinium betaine-based dipolar cycloaddition and a method based on a Mannich type of condensation, followed by Suzuki coupling [12,13]. Suzuki coupling of phenylboronic acid and the three-

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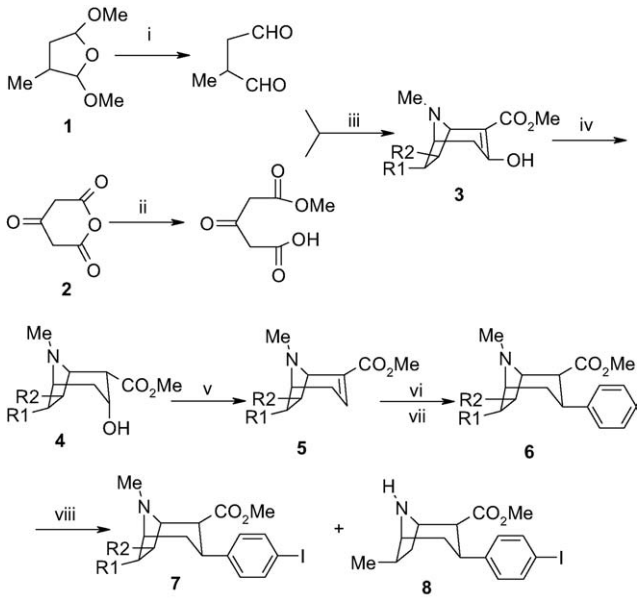
tropinones leads to unsaturated phenyltropanes and reduction of these products yields a complex mixture of isomers. However, the 2β, 3β-orientation of the substituents is known to be important for high affinity binding to monoamine transporters [6,14]. We decided to use the Mannich reaction, but instead of using Suzuki coupling for achieving the phenyltropane function, we used a strategy similar to the synthesis of β-CIT from cocaine [10].

## 2. Chemistry

6/7-Methylsubstituted tropinone analogues of **3** were synthesized by the Mannich reaction resulting in a mixture of **3a** (26%) and **3b** (21%) (Scheme 1). After reduction of **3**, the corresponding tropinols **4a** (82%) and **4b** (88%) were converted to **5a** (38%) and **5b** (48%), respectively, by anti-elimination of water (Scheme 1). The phenyl ring was coupled to the double bond by Michael addition at –40 °C under a nitrogen atmosphere, leading to the corresponding 2β, 3β-configuration of **6a** (41%) and **6b** (31%) as the main product. Compounds **7a** and **7b** were prepared by direct iodination in a mixture of acetic acid and perchloric acid containing mercuric oxide, with yields of 64% and 48%, respectively. The reaction also yielded the demethylated analogue **8**, but only for the 6-*exo*-isomer (yield 19%).

The products were characterized by $^{1}$H and $^{13}$C NMR spectroscopy. Different configurations were identified by 2D-COSY and computational spectroscopic analyses by PERCHit software [15]. The calculated $^{3}J$(2, 3) couplings were compared to the previously published couplings of 2α-carbomethoxy-3β-phenyltropane (α-CPT) and 2β-carbomethoxy-3β-phenyltropane (β-CPT) [16]. Generally, phenyltropanes with a 2α-substituent have $^{3}J$(2, 3) coupling over 10 Hz, whereas 2β-substituted phenyltropanes have the coupling around 5 Hz. Compounds **6a** and **6b** were distinguished by comparing the couplings of the bridge protons to the protons at carbons 1 and 5.




Scheme 1. Synthetic route to the 6/7-methyl-3β-phenyltropanes. Reagents and conditions: (i) 0.1 M HCl, 1 h, 75 °C; (ii) MeOH 1 h r.t.; (iii) $MeNH_2$·HCl, NaOH, citrate buffer, 3 days, r.t.; (iv) $NaBH_4$, –30 °C, MeOH, 3 h; (v) $POCl_3$, pyridine, reflux, 1 h; (vi) PhMgBr, –40 °C, ether, 4 h; (vii) –70 °C, TFA; (viii) HgO, I, $HClO_4$, AcOH, $CH_2Cl_2$, r.t., overnight. **a**: R1 = –Me, R2 = –H, **b**: R1 = –H, R2 = –Me.

## 3. Results and discussion

The affinities ($IC_{50}$) of tropane analogues **6–8** for the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters were determined by in vitro competition studies using [$^{3}$H]-GBR12935, [$^{3}$H]-paroxetine and [$^{3}$H]-nisoxetine, respectively, as radioligands and rat brain regions from striatum, the combined amygdala and hippocampus, and cerebral cortex, respectively [17–19]. For comparison purposes the affinity of (–)-cocaine and β-CIT were also measured. Competition studies were performed at a fixed concentration of the radioligand and a fixed range of four concentrations for the test compounds. The results are summarized in Table 1. When the binding affinities of the reference compounds are compared to affinities from literature, it must be noted that our test procedure, in which [$^{3}$H]-GBR-12935 is used as the ligand, underestimates DAT affinities [10]. Nevertheless, some general features can be discerned. Compounds **6a** and **6b** were almost inactive, and only minor affinities for DAT were observed. In contrast, **7a** and **7b** had affinity for all three transporters, but DAT affinity of **7b** was decreased to a greater extent. The demethylated compound **8** had a similar binding profile as **7b**, but with slightly better affinities. All the tested new compounds had diminished NET affinity.

Locomotor activity of mice was measured after injection of the most active compounds **7a** and **8** as their water soluble hydrochlorides. Hydrochlorides of (–)-cocaine and β-CIT were also tested as reference compounds, in addition to physiological saline solution as a control. Injections were given intraperitoneally (i.p.) and the mice were monitored by a 10 channel IRS Actometer System over a period of 360 min at 10 min intervals. Due to its short duration of action, (–)-

Table 1
Inhibition of [$^{3}$H]-GBR-12935, [$^{3}$H]-paroxetine and [$^{3}$H]-nisoxetine binding at the dopamine, serotonin and norepinephrine transporters, respectively, in rat brain membranes[a]

| Compound | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | DAT | SERT | NET |
| (–)-Cocaine | 3.72 ± 1.22 | 2.39 ± 1.98 | 2.95 ± 0.77 |
| β-CIT | 0.021 ± 0.009 | 0.011 ± 0.007 | 0.045 ± 0.008 |
| **6a** | 27.7 ± 9.5 | NDB | NDB |
| **6b** | 16.9 ± 4.4 | NDB | NDB |
| **7a** | 0.782 ± 0.212 | 0.344 ± 0.087 | 7.49 ± 3.08 |
| **7b** | 4.47 ± 1.56 | 0.402 ± 0.216 | 13.0 ± 2.15 |
| **8** | 2.42 ± 0.57 | 0.260 ± 0.101 | 12.0 ± 1.43 |

[a] Each value is the mean of three independent experiments, each in triplicate. NDB = no displacement of binding.

cocaine was only monitored for 240 min. Cocaine increased locomotor activity dose dependently having a statistically significant effect at 10 and 20 mg/kg (Fig. 1). β-CIT was a very potent motoric stimulator reaching a maximum activity at 0.3 mg/kg ($P < 0.001$ vs. control). Higher doses of β-CIT were catatonic and motility counts were not increased at 1 mg/kg. A single injection of **7a** stimulated locomotor activity equally at 10 and 100 mg/kg ($P < 0.01$ vs. control), nearly as much as 0.3 mg/kg of β-CIT. Injection of **8** (0.2, 2.0 and 20.0 mg/kg) had no effect on locomotor activity as compared to saline treated controls (data not shown).

In conclusion, the present work details a new synthetic route to achieve 6β/7β-methyl-2β-methoxycarbonyl-3β-(4-iodo)phenyltropanes (**7a**, **7b**) and 6β-methyl-2β-methoxycarbonyl-3β-(4-iodo)phenylnortropane (**8**). Competitive in vitro studies showed that C6/C7-substitution decreased the affinity for DAT and SERT, although the affinities were still greater than affinity of cocaine. In addition, affinity to NET was almost abolished. It is obvious that part of the decreased biological activities is due to the fact that all the tested compounds were racemates. In the literature it has been reported that with 6/7-hydroxylated phenyltropanes the C7-position was more favorable for DAT affinity and the both 6-hydroxylated and 7-hydroxylated compounds had notably diminished affinities to SERT [3,6]. However, our results with the 6/7-methyl phenyltropanes were partly the contrary. Methyl substitution at the C6-position was more favorable for DAT and SERT affinity than at the C7-position. In addition, the substitution at the C7-position influenced DAT affinity more than SERT affinity. Demethylation of **7a** (**8**) increased as expected both the affinity and selectivity for SERT [10]. In the locomotor studies in mice, compounds **7a** and **8** evoked totally different behavioral responses, and thus the result supports the in vitro data, because the ability of cocaine analogues to increase locomotor activity is mainly attributable to DAT binding [20–22]. The compound **7a** increased locomotor activity at a wide dose range, while the demethylated analogue **8** had no measurable influence when compared to saline treated controls. However, the compound **7a** was much less potent in increasing the locomotor activity than β-CIT.

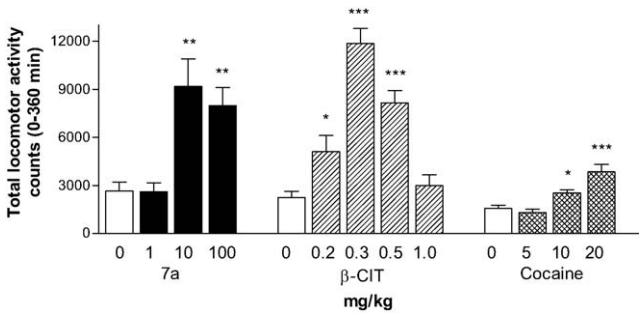



Fig. 1. The total activity counts of **7a**, β-CIT and cocaine in mice. Due to its short duration of action, (–)-cocaine was only monitored for 240 min. Statistics: **7a**: $^{**}P < 0.01$ compared to NaCl treated control animals. Data are mean ± S.E.M. of seven to nine animals; β-CIT: $^{*}P < 0.05$ and $^{***}P < 0.001$. Data are mean ± S.E.M. of 10 animals; cocaine: $^{*}P < 0.05$ and $^{***}P < 0.001$. Data are mean ± S.E.M. of 10 animals.

All the synthesized compounds have no sufficiently high affinities, when use in the PET/SPET is considered. However, the methyl substitution on the C7-position increases the SERT selectivity and thus could offer new possibilities in developing more SERT selective imaging agents.

## 4. Experimental

### 4.1. Chemistry

NMR spectra were performed in $CDCl_3$ on a Bruker 500 MHz spectrometer. TMS was used as internal standard. Reagents in the syntheses were mainly purchased from Aldrich Chemicals. β-CIT was synthesized according to Boja et al. [10] with minor modifications. Column chromatography was carried out on Kieselgel 60.

#### *4.1.1. (±)-6/7-*exo*, 8-dimethyl-3-oxo-8-aza-bicyclo[3.2.1]-octane-2-carboxylic acid methyl ester (**3**)*

Compound **1** (1.46 g, 0.01 mol) was dissolved in HCl (0.1 M, 30 ml) and stirred for 1 h at 75 °C. Compound **2** (1.28 g, 0.01 mol) was dissolved in cold and dry methanol (5 ml) and stirred for 1 h in room temperature (r.t.). Methylamine hydrochloride (1.01 g, 0.015 mol) and NaOH (0.40 g, 0.01 mol) were dissolved in citrate buffer and the stirred mixtures of **1** and **2** were added dropwise. The resulting reaction mixture was stirred for 3 days at r.t. The mixture was acidified with HCl and extracted three times with dichloromethane. The aqueous layer was basified (pH 12) with conc. $NH_4OH$ and extracted three times with dichloromethane. The combined organic fractions were dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The residue was dissolved in diethyl ether and filtered. The filtrate was evaporated to dryness and dissolved in hot hexane, filtered and evaporated to dryness. The product was purified with column chromatography using EtOAc/MeOH/$NH_4OH$ (95:5:0.2). Yields for 6-*exo*-isomer (**3a**, $R_f = 0.46$) and 7-*exo*-isomer (**3b**, $R_f = 0.62$) were 0.55 g (26%) and 0.45 g (21%), respectively. **3a**: $^1$H NMR ($CDCl_3$) $\delta$ 11.79 (bs, 1H), 3.79 (d, $J$ = 5.5 Hz, 1H), 3.76 (s, 3H), 2.90 (m, 1H), 2.71 (dd, $J$ = 18.6 Hz, 4.8 Hz, 1H), 2.36 (s, 3H), 2.05–1.94 (m, 2H), 1.87 (d, $J$ = 18.6 Hz, 1H), 1.63, (m, 1H), 1.14 (d, $J$ = 6.7 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 171.8, 169.2, 100.8, 64.1, 58.2, 51.4, 44.6, 38.9, 34.8, 32.1, 22.1. **3b**: $^1$H NMR ($CDCl_3$) $\delta$ 11.72 (bs, 1H), 3.77 (s, 3H), 3.39 (bs, 1H), 3.37 (m, 1H), 2.68 (ddd, $J$ = 18.8 Hz, 4.9 Hz, 2.1 Hz, 1H), 2.35 (s, 3H), 2.17 (m, 1H), 1.82–1.75 (m, 2H), 1.67 (m, 1H), 1.11 (d, $J$ = 7.3 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 172.0, 169.1, 101.0, 62.9, 57.1, 51.4, 43.3, 39.9, 34.1, 31.0, 22.6.

#### *4.1.2. (±)-6/7-*exo*, 8-dimethyl-3-hydroxy-8-aza-bicyclo-[3.2.1]octane-2-carboxylic acid methyl ester (**4**)*

$NaBH_4$ (0.62 g, 0.016 mol) was dissolved in methanol (10 ml) under a nitrogen atmosphere. **3** (0.55 g, 0.003 mol) was dissolved in methanol (26 ml) and added dropwise at

–30 °C. The reaction mixture was stirred at –30 °C for 3 h. Concentrated HCl (1.34 ml) was carefully added dropwise and the reaction mixture was allowed to warm to r.t. and evaporated to dryness. The residue was dissolved in water and basified with conc. $NH_4OH$. Solid NaCl was added and the mixture was extracted with $CHCl_3$. The combined organic fractions were dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. Yields for 6-*exo*-isomer (**4a**) and 7-*exo*-isomer (**4b**) were 0.52 g (82%) and 0.56 g (88%), respectively. $^1$H NMR spectra were not analyzed for compounds **4a** and **4b**, due to broad signals caused by rapid dynamical changes of the tropane ring conformations. **4a**: $^1$H NMR ($CDCl_3$) $\delta$ 4.32 (m, 1H). $^{13}$C NMR ($CDCl_3$) $\delta$ 175.3, 64.9, 64.9, 61.1, 51.9, 43.0, 36.0, 35.9, 35.8, 31.1, 23.4. [M + H$^+$] for $C_{11}H_{19}NO_3$ calc. 214.14377, found 214.14167. **4b**: $^1$H NMR ($CDCl_3$) $\delta$ 4.32 (m, 1H). $^{13}$C NMR ($CDCl_3$) $\delta$ 175.2, 67.3, 64.9, 58.7, 51.8, 43.9, 37.7, 36.1, 34.5, 31.3, 23.6.

*4.1.3. (±)-6/7-*exo*, 8-dimethyl-8-aza-bicyclo[3.2.1]oct-2-ene-2-carboxylic acid methyl ester (**5**)*

Compound **4** (0.52 g, 0.002 mol) was dissolved in pyridine (10 ml) under nitrogen atmosphere, and $POCl_3$ (4.8 ml) was added. The reaction mixture was refluxed for 1 h and evaporated to dryness. The residue was dissolved in water and acidified with conc. HCl, extracted with diethyl ether and basified with conc. $NH_4OH$. The basic water fraction was extracted with dichloromethane and the combined organic layers were dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography using EtOAc/MeOH (8:2). Yields for 6-*exo*-isomer (**5a**, $R_f$ = 0.50) and 7-*exo*-isomer (**5b**, $R_f$ = 0.56) were 0.18 g (38%) and 0.23 g (48%), respectively. **5a**: $^1$H NMR ($CDCl_3$) $\delta$ 6.83 (m, 1H), 3.81 (d, $J$ = 5.9 Hz, 1H), 3.73 (s, 3H), 2.81 (d, $J$ = 4.2 Hz, 1H), 2.56 (dt, $J$ = 19.8 Hz, 3.9 Hz, 1H), 2.35 (s, 3H), 2.08 (dd, $J$ = 11.6 Hz, 8.5 Hz, 1H), 1.96 (m, 1H), 1.80 (dd, $J$ = 19.8 Hz, 4.2 Hz, 1H), 1.67 (m, 1H), 1.14 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 166.7, 135.7, 132.8, 63.6, 59.4, 51.6, 44.5, 39.4, 34.7, 29.4, 22.1. [M + H$^+$] for $C_{11}H_{17}NO_2$ calc. 196.13321 found 196.13234. **5b**: $^1$H NMR ($CDCl_3$) $\delta$ 6.81 (m, 1H), 3.74 (s, 3H), 3.37 (s, 1H), 3.24 (m, 1H), 2.53 (dm, $J$ = 19.8 Hz, 1H), 2.34 (s, 3H), 2.21 (m, 1H), 1.79–1.64 (m, 3H), 1.14 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 167.0, 135.3, 132.8, 64.3, 56.5, 51.5, 43.5, 40.7, 34.3, 28.6, 22.6. [M + H$^+$] for $C_{11}H_{17}NO_2$ calc. 196.13321, found 196.13234.

*4.1.4. (±)-6/7-*exo*, 8-dimethyl-3-phenyl-8-aza-bicyclo-[3.2.1]octane-2-carboxylic acid methyl ester (**6**)*

Compound **5** (0.18 g, 0.92 mmol) was dissolved in diethyl ether (10 ml) and the mixture was cooled to –40 °C. PhMgBr (3 M in diethyl ether, 0.60 ml, 1.80 mmol) was added dropwise with vigorous stirring. The reaction mixture was stirred at –40 °C for 4 h. The mixture was cooled to –70 °C and TFA (0.37 g) was added. The reaction mixture was allowed to warm to –5 °C and 10 ml of water was added. The mixture was acidified with conc. HCl and extracted with diethyl ether. The water layer was basified with conc. $NH_4OH$ and extracted with $CHCl_3$. The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography using diethyl ether/TEA (95:5). Yields for 6-*exo*-isomer (**6a**, $R_f$ = 0.95) and 7-*exo*-isomer (**6b**, $R_f$ = 0.97) were 103.1 mg (41%) and 68.0 mg (31%), respectively. **6a**: $^1$H NMR ($CDCl_3$) $\delta$ 7.28–7.21 (m, 4H), 7.16 7.12 (m, 1H), 3.69 (dm, $J$ = 6.2 Hz, 1H), 3.45 (s, 3H), 3.11–3.03 (m, 2H), 2.88 (m, 1H), 2.55 (m, 1H), 2.49 (s, 3H), 2.19 (m, 1H), 2.05 (m, 1H), 1.94 (m, 1H), 1.70 (m, 1H), 1.25 (d, $J$ = 7.1 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 172.4, 143.3, 127.9, 127.3, 125.8, 69.4, 66.2, 52.1, 51.0, 42.7, 37.3, 36.3, 34.5, 33.5, 24.1. [M + H$^+$] for $C_{17}H_{23}NO_2$ calc. 274.18016, found 274.17958. **6b**: $^1$H NMR ($CDCl_3$) $\delta$ 7.28–7.21 (m, 4H), 7.16–7.12 (m, 1H), 3.48 (s, 3H), 3.46 (m, 1H), 3.31 (bs, 1H), 3.06 (m, 1H), 2.95 (m, 1H), 2.54 (td, $J$ = 12.6 Hz, 2.7 Hz, 1H), 2.47 (s, 3H), 2.29 (m, 1H), 1.94 (m, 1H), 1.84 (m, 1H), 1.63 (m, 1H), 1.29 (d, $J$ = 7.5 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 172.3, 143.4, 127.9, 127.2, 125.7, 72.3, 63.1, 52.7, 51.0, 42.5, 38.2, 35.6, 34.4, 32.6, 24.4. [M + H$^+$] for $C_{17}H_{23}NO_2$ calc. 274.18016, found 274.18037.

*4.1.5. (±)-3-(4-iodo-phenyl)-6/7-*exo*, 8-dimethyl-8-aza-bicyclo[3.2.1]octane-2-carboxylic acid methyl ester (**7**) and 3-(4-iodo-phenyl)-6-*exo*-methyl-8-aza-bicyclo[3.2.1]-octane-2-carboxylic acid methyl ester (**8**)*

Compound **6** (103.1 mg, 0.38 mmol) was dissolved in a solution, that contained 1.3 ml acetic acid, 0.4 ml 70% perchloric acid and mercury(II)oxide (107.3 mg, 0.50 mmol). A solution of iodine (226.3 mg, 0.89 mmol), 3.3 ml dichloromethane and 1.7 ml acetic acid was added to the reaction mixture. The mixture was stirred overnight at r.t. and filtered. The filtrate was eluted into 1.7 ml of water and 3.3 ml of dichloromethane and cooled to 0 °C. The mixture was basified slowly with $NH_4OH$. The layers were separated and the water layer was extracted four times with dichloromethane. The combined organic fractions were dried over $Na_2SO_4$, filtered and evaporated to dryness. The residue was dissolved to dichloromethane and precipitated with diethyl ether/TEA (1:1). The mixture was centrifuged and the supernatant was removed. The precipitating procedure was repeated four times. The dichloromethane solution was evaporated to dryness and purified by column chromatography with EtOAc/MeOH/$NH_3$ (9:1:0.01). Yields for 6-*exo*-isomer (**7a**, $R_f$ = 0.78), 7-*exo*-isomer (**7b**, $R_f$ = 0.89) and **8** ($R_f$ = 0.48) were 97.1 mg (64%), 72.8 mg (48%) and 27.8 mg (19%), respectively. **7a**: $^1$H NMR ($CDCl_3$) $\delta$ 7.57 (d, $J$ = 8.4 Hz, 2H), 7.00 (d, 2H), 3.69 (m, 1H), 3.48 (s, 3H), 3.09 (bs, 1H), 2.99 (m, 1H), 2.83 (m, 1H), 2.49 (m, 1H), 2.48 (s, 3H), 2.16 (m, 1H), 2.03 (m, 1H), 1.94 (m, 1H), 1.66 (m, 1H), 1.24 (d, $J$ = 7.4 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 172.3, 142.8, 137.0, 129.4, 91.2, 69.3, 66.2, 51.7, 51.3, 42.7, 37.1, 36.0, 34.2, 33.3, 24.0. [M + H$^+$] for $C_{17}H_{22}NO_2I$ calc. 400.07680, found 400.07543. **7b**: $^1$H NMR ($CDCl_3$) $\delta$ 7.57 (d, $J$ = 8.5 Hz, 2H), 6.99 (d, 2H), 3.50 (s, 3H), 3.44 (m, 1H), 3.31 (m, 1H), 2.99 (m, 1H), 2.90 (m, 1H), 2.48 (m, 1H), 2.46 (s, 3H), 2.26 (m, 1H), 1.93 (m, 1H), 1.84 (m,

1H), 1.59 (m, 1H), 1.29 (d, $J$ = 7.4 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 172.2, 143.1, 136.9, 129.4, 91.0, 69.2, 66.2, 51.8, 51.2, 42.7, 37.3, 36.1, 34.1, 33.4, 24.1. [M + H$^+$] for $C_{17}H_{22}NO_2I$ calc. 400.07680, found 400.07543. **8**: $^1$H NMR ($CDCl_3$) $\delta$ 7.59 (d, $J$ = 8.4 Hz, 2H), 6.95 (d, 2H), 3.72 (m, 1H), 3.41 (s, 3H), 3.23 (m, 1H), 3.13 (m, 1H), 2.69 (m, 1H), 2.34 (td, $J$ = 12.7 Hz, 3.0 Hz, 1H), 2.11 (m, 1H), 2.00 (m, 1H), 1.66–1.57 (m, 2H), 1.14 (d, $J$ = 7.1 Hz, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 173.4, 142.0, 137.4, 129.5, 91.8, 63.6, 55.1, 51.3, 50.8, 37.3, 36.9, 35.7, 32.8, 22.9. [M + H$^+$] for $C_{16}H_{20}NO_2I$ calc. 386.06115, found 386.06139.

### 4.2. In vitro-assays

#### 4.2.1. Membrane preparation for in vitro assays

Wistar rats were decapitated and the heads were dropped in liquid nitrogen for 2–3 s. The brains were quickly removed and dissected on ice, into striatum, the combined amygdala and hippocampus regions and cerebral cortex. The tissues were kept frozen at –70 °C until homogenized and membranes prepared for binding studies. Membrane preparations were used within 2 weeks after preparation. Protein concentrations of the homogenized tissues were assayed in principle according to method of Lowry et al. [23].

#### 4.2.2. Dopamine transporter assay

DAT competition studies were performed according to the method of Andersen [17] with some modifications. Striatal tissue of five to six rats (0.3–0.4 g) was homogenized in 2 × 10 ml of ice cold 50 mM Tris–citrate buffer, pH 7.4, containing 120 mM of NaCl and 4 mM of $MgCl_2$, using an ultra-turrax homogenizer. The buffer was added to the homogenate to make a volume 500 times the original weight of the tissue. The homogenate was centrifuged at 30,000 × $g$ for 10 min at +4 °C. The pellet was resuspended in 500 × volume (original tissue weight) of 50 mM Tris–citrate buffer. The homogenate was centrifuged at 30,000 × $g$ for 10 min and +4 °C. The pellet was resuspended in 300 × volume (original tissue weight) of 50 mM Tris–citrate buffer. The membrane suspension was divided into aliquots and stored frozen at –80 °C until used.

The assay sample contained 0.4 ml of tissue suspension (protein about 0.2 mg/ml) in a total volume of 0.5 ml. [$^3$H]-GBR-12935, specific activity 53.5 Ci/mmol, was used at 1 nM concentration for displacement studies. (–)-Cocaine and β-CIT were used as a reference compounds over five to seven concentrations ranging from 0.1 nM to 10 μM. Non-specific binding was determined in the presence of 1 μM of GBR-12909. Compounds **6**–**8** were tested at four concentrations ($10^{-4}$–$10^{-7}$ M) in triplicate. Samples were incubated for 60 min at 0 °C and filtered through Filtermat B (Wallac) glass fiber filters pretreated with 0.1% bovine serum albumin, using a cell harvester (Brandel, Gaithersburg, MD, USA). The filters were rapidly washed with ice cold 0.9% NaCl and allowed to dry over night at r.t. The filters were coated with Meltilex™ B/HS melt-on scintillator sheets (Wallac, Perkin Elmer) and the radioactivity was measured with a Micro-Beta 1450 trilux Wallac liquid scintillation and luminescence counter with 22% counting efficiency.

#### 4.2.3. Serotonin transporter assay

The affinities of the compounds to SERT were studied using membranes from the combined amygdala and hippocampus regions. The membranes were prepared and the displacement studies performed according to Habert et al. [18]. Briefly, tissue was homogenized in 50 volumes of buffer (50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 5 mM KCl) twice for 10 s using an ultra-turrax homogenizer. The homogenate was centrifuged at 30,000 × $g$ for 10 min and the pellet resuspended in 50 volumes of buffer and centrifuged once more at 30,000 × $g$ for 10 min. The pellet was resuspended in the same buffer to make a protein concentration of 3–5 mg/ml. The membrane suspension was either used immediately or frozen in liquid nitrogen and kept in –80 °C for 2 weeks at most.

The assay sample contained 0.8 ml of tissue suspension in a total volume of 1.0 ml. [$^3$H]-Paroxetine, specific activity 17.1 Ci/mmol, was used at 1 nM concentration for displacement studies. (–)-Cocaine and β-CIT were used as a reference compounds over five to seven concentrations ranging from 0.1 nM to 10 μM. Non-specific binding was determined in the presence of 1 μM of fluoxetine. Compounds **6**–**8** were tested at four concentrations ($10^{-4}$–$10^{-7}$ M) in triplicate. Samples were incubated at r.t. for 60 min and filtered through glass fiber filters (Filtermat B) pretreated with 0.05% polyethylenimine, and harvested as described for DAT. The filters were rapidly washed with ice cold incubation buffer and prepared for radioactivity measurement as described above.

#### 4.2.4. Norepinephrine transporter assay

NET binding studies were performed according to Tejani-Butt [19]. Cerebral cortex was homogenized with an ultra-turrax for 15 s in 30 volumes of ice cold buffer (50 mM Tris, pH 7.4, 120 mM NaCl, 5 mM KCl). The homogenate was centrifuged at 40,000 × $g$ for 10 min and +4 °C. The pellet was resuspended in 30 volumes of buffer and centrifuged as before. This phase was repeated twice. The final pellet was suspended in 30 volumes of ice cold incubation buffer (50 mM Tris, pH 7.4, 300 mM NaCl, 5 mM KCl).

The reaction mixture consisted of 0.4 ml of membrane preparation (0.3–0.55 mg of protein) in a final volume of 0.5 ml. [$^3$H]-Nisoxetine, specific activity 85 Ci/mmol, at 1 nM final concentration was used for the displacement studies. Desipramine (1 μM) was used to define the non-specific binding. (–)-Cocaine and β-CIT were used as reference compounds at five to seven concentrations ranging from 0.1 nM to 10 μM. Compounds **6**–**8** were tested at four concentrations ($10^{-4}$–$10^{-7}$ M) in triplicate. The samples were incubated for 4 h at +4 °C in a shaker. The reaction was terminated by rapidly filtering through Wallac-B glass fiber filter. Ice cold incubation buffer was used to rinse the nonbound radioactivity from the filters. The filters were treated and the radioactivity counted as above for DAT.

### 4.3. Locomotor activity

#### 4.3.1. Animals for locomotor activity measurements

Male C57BL/6J mice were used to assess locomotor activity. Prior to testing, animals were kept under standard conditions (temperature 22 ± 1 °C), in small groups (3–12 mice per group), at a 12 h dark/light cycle (lights on at 07:00 h). They had free and continuous access to fresh tap water and food pellets (Lactamin R36, Lactamin AB Södertälje, Sweden). The experiments were carried out during the daylight hours.

#### 4.3.2. Locomotor activity testing procedure

The animals were housed individually in test cages (42.5 × 25 × 15 cm) and allowed to acclimate to their surroundings overnight. Food and water were available ad libitum. The following morning, mice were injected intraperitoneally with vehicle (NaCl) or test compounds in a volume of 10 ml/kg. Activity was measured using a 10 channel IRS Actometer System, at 10 min consecutive periods for a total of 360 min post-injection for **7a**, **8** and β-CIT, and 240 min for (–)-cocaine. The total locomotor activity was recorded by measuring the movements of the heat radiation source (i.e. mouse). For this assay, data is represented as motility (0–100) during each motility integration time (10 min). The area underneath the mobility curve (AUC) was used to estimate the effect of the test compounds ($AUC_{0–300\ min}$) on locomotor activity. The statistical significance of any effect was tested using one-way ANOVA followed by Bonferroni's modification of the Newman–Keuls test.

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