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# A novel spirocyclic tropanyl-$\Delta^2$-isoxazoline derivative enhances citalopram and paroxetine binding to serotonin transporters as well as serotonin uptake

Clelia Dallanoce [a,*], Mara Canovi [b], Carlo Matera [a], Tiziana Mennini [b], Marco De Amici [a], Marco Gobbi [b], Carlo De Micheli [a]

[a] Dipartimento di Scienze Farmaceutiche, Sezione di Chimica Farmaceutica 'Pietro Pratesi', Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[b] Dipartimento di Biochimica e Farmacologia Molecolare, Istituto di Ricerche Farmacologiche 'Mario Negri', Via La Masa 19, 20156 Milano, Italy

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ABSTRACT

A group of spirocyclic tropanyl-$\Delta^2$-isoxazolines was synthesized exploiting the 1,3-dipolar cycloaddition of nitrile oxides to olefins. Their interaction with the dopamine and serotonin transporters (DAT and SERT, respectively) was evaluated through binding experiments. The majority of the compounds had no inhibitory effects ($IC_{50}$ >> 10 μM), while some had an $IC_{50}$ value in the range 5–10 μM (**8a–c**, **10b** and **11c** on DAT, **12b** on SERT). Unexpectedly, one of the tertiary amines under investigation, that is 3′-methoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole **7a**, was able to enhance at a concentration of 10 μM both [$^3$H]citalopram and [$^3$H]paroxetine binding to SERT in rat brain homogenate (up to 25%, due to an increase of $B_{max}$) and [$^3$H]serotonin uptake (up to 30%) in cortical synaptosomes. This peculiar pharmacological profile of **7a** suggests it binds to an allosteric site on SERT, and positions derivative **7a** as a very useful tool to investigate SERT machinery.



## 1. Introduction

The protein transporters responsible for the synaptic reuptake of neurotransmitters play an essential role in the control of neuronal signaling in the central nervous system (CNS).[1] Within them, the transporters which regulate the reuptake of monoamines serotonin, dopamine and norepinephrine (SERT, DAT, and NET, respectively) are involved in several neurological conditions, including depression and drug abuse, and are relevant targets for psychostimulants and therapeutic drugs used in the treatment of mood disorders.[2] In particular, depression, a mood disorder which represents a growing global health problem, has been related to abnormal low levels of biogenic amines in the CNS.[3] Thus, most antidepressants currently available for clinical practice share a similar mode of action, which involves the modulation of monoaminergic synaptic transmission through either a selective inhibition (e.g., citalopram, a selective serotonin reuptake inhibitor, SSRI) or a nonselective inhibition (e.g., duloxetine, a serotonin/noradrenaline reuptake inhibitor, SNRI) of transporter systems.[4–6]

Cocaine **1** (Fig. 1) is an alkaloid isolated from the Peruvian *Erythroxylon coca* plant endowed with psychoactive activity,[7] which inhibits the monoamine transporters at submicromolar concentrations.[8] The reinforcing and stimulatory activity of **1** are however mainly related to the inhibition of synaptic reuptake of dopamine (DA) and, to a lesser extent, of serotonin (5-HT), triggered by the binding of cocaine to DAT and SERT, respectively.[9,10] Moreover, several results from behavioral and neuroimaging studies supported the involvement of DAT in the neuropharmacological and addictive properties of cocaine.[1] As far as the structure–activity relationships of cocaine-related derivatives are concerned, several modifications of the pharmacophoric elements of **1** have been explored, which affect the molecular recognition and may address the new ligands towards a specific monoamine transporter. As an example, a variation of the substitution pattern in position 3 (i.e., the 3β-phenyl tropane **2a** and its substituted analogues **2b–d**, Fig. 1) brought about an improvement of binding affinity at DAT over SERT and NET.[11–14] In particular, [$^{125}$I]**2d** has been proposed as a high-affinity photolabel compound to study the DAT protein structure.[14] On the other hand, compound **3** (Fig. 1), a tropane-containing analogue of the antidepressant fluoxetine, a selective serotonin reuptake inhibitor (SSRI), showed a selectivity profile similar to that of its parent derivative.[12]

In this study, we aimed at extending the investigation of the biological properties of compounds incorporating the tropane ring system, a moiety that we previously used to prepare derivatives structurally related to cytisine, a naturally occurring nicotinic agonist.[15] To this end, we designed the group of analogues **4a–12a** as potential ligands for DAT and SERT. In the new compounds, the 8-methyl-8-azabicyclo[3.2.1]octane skeleton, which characterizes

* Corresponding author. Tel.: +39 02 50319327; fax: +39 02 50319326.
*E-mail address:* clelia.dallanoce@unimi.it (C. Dallanoce).

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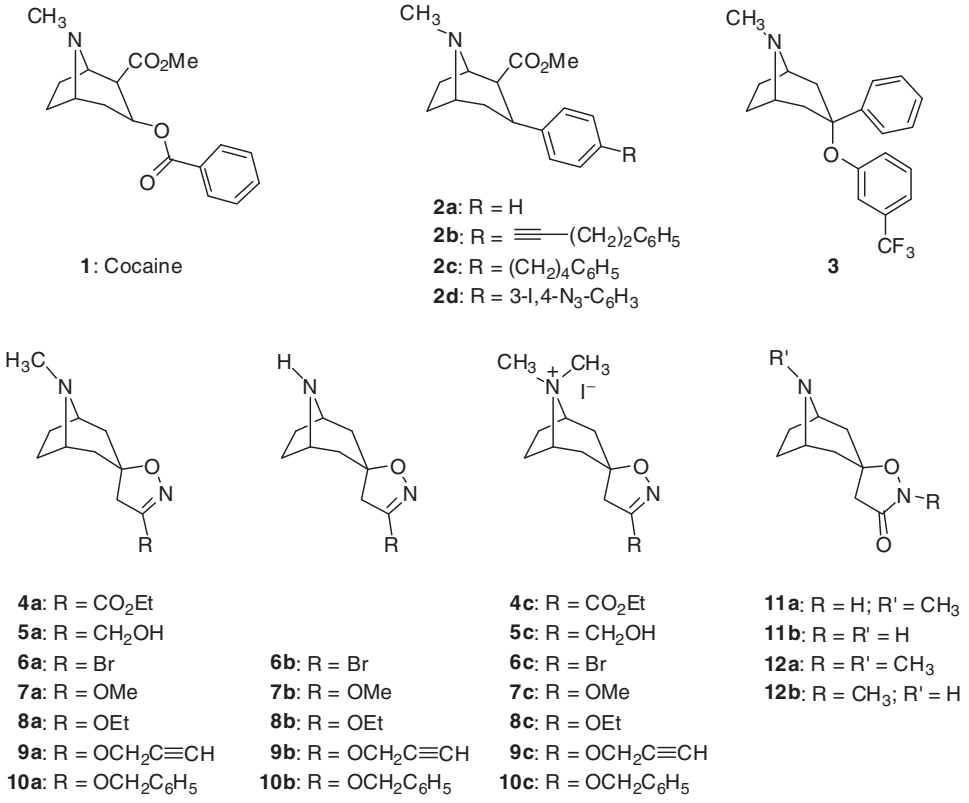



**Figure 1.** Structures of model (**1–3**) and target (**4–12**) compounds in this investigation.

cocaine, is spiro-condensed with different 3-substituted $\Delta^2$-isoxazolines (**4a–10a**) or 2-substituted isoxazolidin-3-ones (**11a**, **12a**). We also prepared and tested the corresponding nortropane derivatives **6b–12b** and the quaternary dimethylammonium salts **4c–10c**. The target derivatives were first assessed for binding affinity at rat striatal DAT and rat cortical SERT, then we evaluated their ability to modify the 5-HT uptake in cortical synaptosomal preparations.

## 2. Results and discussion

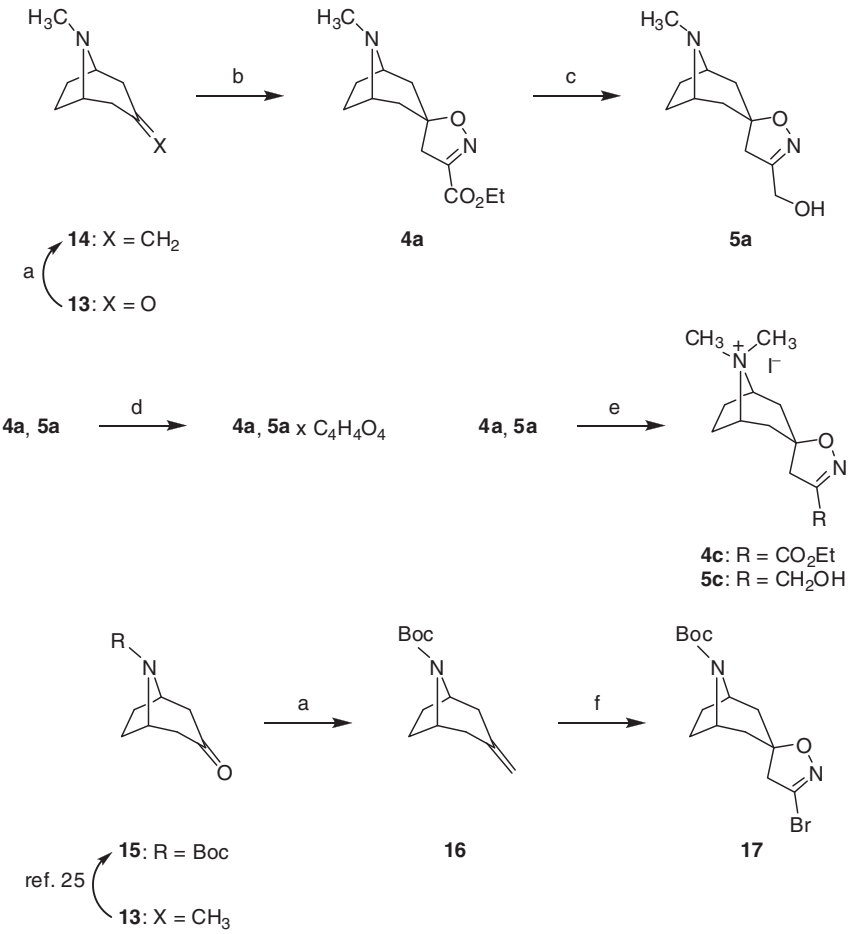
### 2.1. Chemistry

$\Delta^2$-Isoxazolines and isoxazoles with various functionalization patterns are among the most interesting bioactive compounds that we have investigated.[16–18] The target heterocycles have been frequently prepared using the 1,3-dipolar cycloaddition of nitrile oxides to alkenes as the key step, an approach which allowed us to achieve novel derivatives provided with different pharmacological profiles.[19–21] In the present investigation, the alkenes we used as dipolarophiles were the two *N*-substituted-3-methylene-8-azabicyclo[3.2.1]octanes **14** and **16** (Scheme 1). The tertiary amine **14**,[22] easily prepared from the commercially available tropinone **13**, was reacted with excess ethoxycarbonylformonitrile oxide, generated in situ with sodium bicarbonate from its stable precursor 2-chloro-2-(hydroximino)-acetate.[23] The reaction afforded the desired cycloadduct **4a** in a poor yield (13%), and its ester function was reduced with sodium borohydride to give the corresponding primary alcohol **5a** (Scheme 1). The two tertiary amines **4a** and **5a** were then treated with fumaric acid or excess iodomethane to provide the related crystalline fumarates or dimethylammonium iodides **4c** and **5c**, respectively.

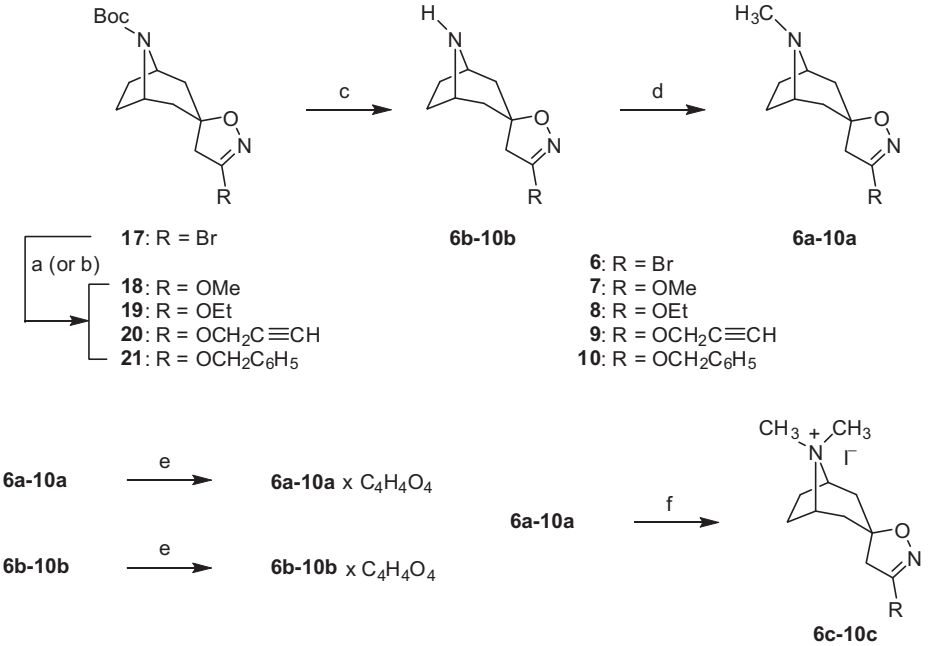
To improve the yield of the cycloaddition step, we prepared the *N*-Boc alkene **16**, already described in the patent literature.[24] Following a known protocol,[25] tropinone **13** was transformed into ketone **15** which was then submitted to a Wittig reaction to give olefin **16** in high yield. Cycloaddition of **16** to bromonitrile oxide[26] afforded the expected spirocyclic 3-bromo-$\Delta^2$-isoxazoline **17** in 82% yield (Scheme 1). The latter intermediate underwent a smooth nucleophilic displacement of bromine by some alcohols, in the presence of a base (potassium carbonate or a sodium hydride suspension), to afford the corresponding alkoxy-$\Delta^2$-isoxazolines **18–21** in 72–90% yield (Scheme 2). The *N*-Boc intermediates **17**–**21** were treated with a dichloromethane solution of trifluoroacetic acid to provide secondary amines **6b**–**10b**, which were then transformed into the corresponding tertiary analogues **6a**–**10a** by reaction with aqueous formaldehyde and sodium borohydride. The two groups of derivatives **6a–10a** and **6b–10b** were converted into the corresponding crystalline fumarates, and quaternary dimethylammonium salts **6c–10c** were also obtained from **6a–10a** (Scheme 2).

Worth noting, both pericyclic reactions to alkenes **14** and **17** afforded only one of the two diastereomeric forms. We chose the fumarate of **7a** to perform a more detailed NMR analysis (see Supplementary data) by means of the 2D COSY, HSQC and NOESY techniques. Our results indicated that 3′-methoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate **7a**, and hence all the spirocyclic derivatives herein described, has the (1*R*,3*s*,5*S*)-configuration, that is the $\Delta^2$-isoxazoline oxygen occupies the equatorial β-position relative to the tropane bridgehead nitrogen.

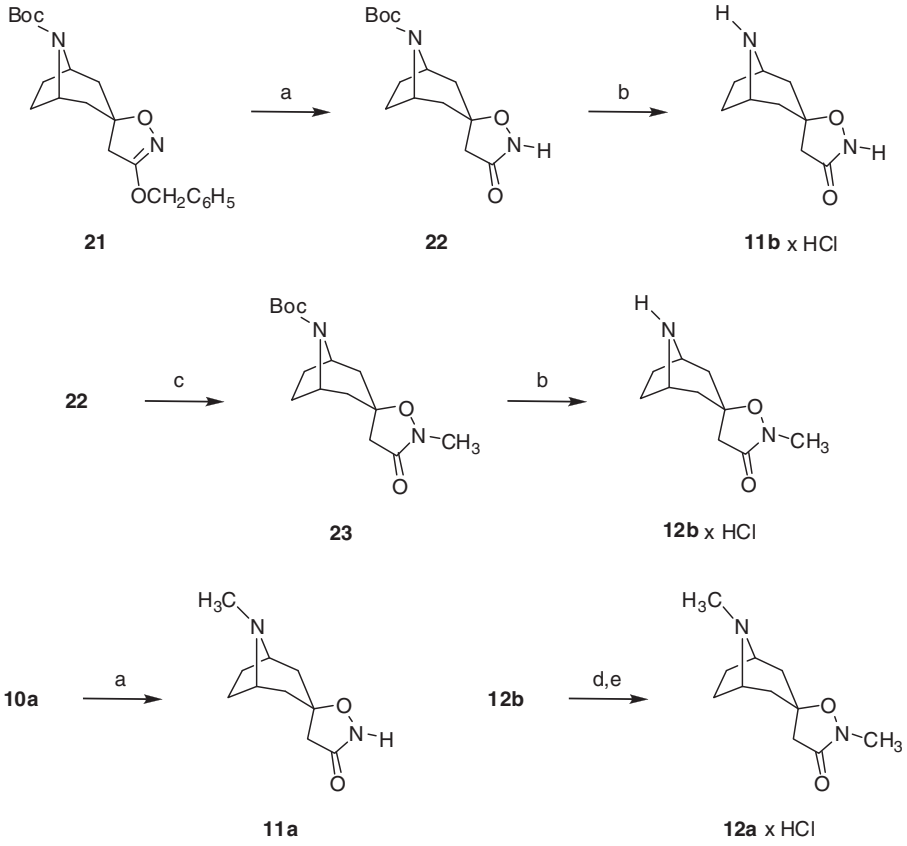
As depicted in Scheme 3, a standard catalytic hydrogenation of 3-benzyloxy-$\Delta^2$-isoxazolines **21** and **10a** produced cyclic carbamates **22** and **11a** in high yield. Intermediate spirocyclic isoxazolidin-3-one **22** was then transformed into its *N*-methyl analogue **23** by reaction with iodomethane in an acetone suspension of potassium carbonate. Removal of the *N*-Boc-protecting group from **22** and **23** gave **11b** × **HCl** and **12b** × **HCl**, respectively. Finally, tertiary amine **12a**




**Scheme 1.** Reagents and conditions: (a) $CH_3P(C_6H_5)_3Br$ (1.4 equiv), *tert*-BuOK, THF, rt, 1 h; (b) $Et_2O_2CC(Cl)=NOH$ (4 equiv), $NaHCO_3$ (10 equiv), dioxane, reflux, 2 d; (c) $NaBH_4$ (3 equiv), EtOH, rt, 3 h; (d) $C_4H_4O_4$ (1.1 equiv), MeOH, rt, 12 h; (e) $CH_3I$ (10 equiv), MeOH, rt, 3 h; (f) $Br_2C=NOH$ (1.2 equiv), $NaHCO_3$, AcOEt, rt, 12 h.




**Scheme 2.** Reagents and conditions: (a) MeOH or EtOH, $K_2CO_3$ (10 equiv), reflux, 2 h; (b) NaH, $HC\equiv CCH_2OH$ (4 equiv) or $C_6H_5CH_2OH$ (6 equiv), THF, 0 °C → reflux, 3 h; (c) $CF_3CO_2H$ (10 equiv), $CH_2Cl_2$, 0 °C → rt, 3–18 h; (d) 37% aq HCHO (5 equiv), MeOH, $NaBH_4$ (5 equiv), rt, 2–10 h; (e) $C_4H_4O_4$ (1.1 equiv), MeOH, rt, 12 h; (f) $CH_3I$ (10 equiv), MeOH, rt, 3 h.




**Scheme 3.** Reagents and conditions: (a) $H_2$, 10% Pd/C, MeOH, 1 atm, 6 h; (b) 4 N HCl (8.7 equiv), dioxane, 0 °C, 2 h; (c) $CH_3I$ (7 equiv), $K_2CO_3$, acetone, rt, 16 h; (d) 37% aq HCHO (5 equiv), MeOH, $NaBH_4$ (5 equiv), rt, 7 h; (e) 2 N HCl (8 equiv), $Et_2O$, 0 °C, 2 h.

was obtained from **12b** through a standard reductive amination, then isolated as its crystalline hydrochloride (Scheme 3).

### 2.2. Pharmacology

We evaluated the in vitro binding of the synthesized compounds to DAT and SERT using as selective radioligands [$^3$H]WIN 35,428 and [$^3$H]citalopram, respectively. Derivatives **4a–10a** and **6b–10b** were assayed as fumarates while **11b**, **12a**, and **12b** were tested as hydrochlorides, and **11a** as free base. Figure 2a shows the effects of the compounds, tested at 10 μM, on specific [$^3$H]WIN 35,428 binding to DAT of rat brain cortex. Most compounds inhibited the binding by less than 50%, indicating $IC_{50}$ values higher than 10 μM. Since the three 3-ethoxy-substituted-$\Delta^2$-isoxazolines **8a–c** and the two secondary amines **10b** and **11b** showed some inhibitory activity, further concentration-dependent experiments were carried out (not shown); their $IC_{50}$ values were all in the 5–10 μM range. Figure 2b shows the specific binding of [$^3$H]citalopram to SERT of rat brain cortex, measured in the absence or presence of the test compounds at 10 μM. No significant inhibition was detected for any of these compounds, with the exception of the isoxazolidin-3-one **12b**, whose $IC_{50}$ value was then estimated as 5 μM. Worth noting, we observed an unexpected increase of [$^3$H]citalopram binding induced by the 3-methoxy-tropanyl-$\Delta^2$-isoxazoline **7a**, which was consistently detected in five independent binding experiments, and amounted to 25 ± 5% (mean ± S.E.M., $n$ = 16, from five different experiments, $p$ <0.01, vs vehicle). Importantly, no effect of **7a** was found on the non-specific binding of [$^3$H]citalopram (not shown), which implies that derivative **7a** is actually increasing binding to SERT. A comparable behavior was also detected for the 3-ethoxy-tropanyl-$\Delta^2$-isoxazoline **8a**, the closest structural analogue of **7a**, which caused a lower (11 ± 3%), but still significant, enhancement of [$^3$H]citalopram binding (mean ± S.E.M., $n$ = 16, from three different experiments, $p$ <0.05, vs vehicle).

When tested at 10 μM, **7a**, but not any other compound, increased the SERT-mediated [$^3$H]5-HT uptake in rat brain cortex synaptosomes (+30 ± 8%, $p$ <0.05, $n$ = 4) (Fig. 3). As illustrated in Figure 4a and b, the effects of **7a** on both [$^3$H]citalopram binding and [$^3$H]5-HT uptake are concentration-dependent, with a bell shape: the highest increase of binding/uptake was induced at a concentration of 10–30 μM, with lower effects at higher concentrations. It might be suggested that this finding is the result of two opposite effects of the compound: (i) a potentiating allosteric effect at lower concentrations (10–30 μM), and (ii) an inhibitory orthosteric effect exerted at higher concentrations (>30 μM), which counteracts the former one. The same analysis performed on the ethoxy substituted analogue **8a** (Fig. 4c and d) showed a qualitatively similar profile, although the enhancing effects of **8a** were comparably less pronounced than those of the methoxy derivative **7a**.

The properties displayed at SERT by **7a** seem to be related to rather strict molecular features: (i) the enhancing effects require the presence of the *N*-methyl group on the tropane moiety, and (ii) more surprisingly, the apparent overriding influence of the size of the substituents, see methoxy versus ethoxy, on the 3 position of the $\Delta^2$-isoxazoline ring, which significantly affects the enhancement of [$^3$H]citalopram binding and [$^3$H]5-HT uptake of tertiary bases, both effects being maximized by the methoxy group. On the other hand, the isoxazolidin-3-one derivative **12a**, which is a positional isomer of $\Delta^2$-isoxazoline **7a** also characterized by the *N*-methyl tropane ring, did not show the same enhancing effect on [$^3$H]citalopram binding.

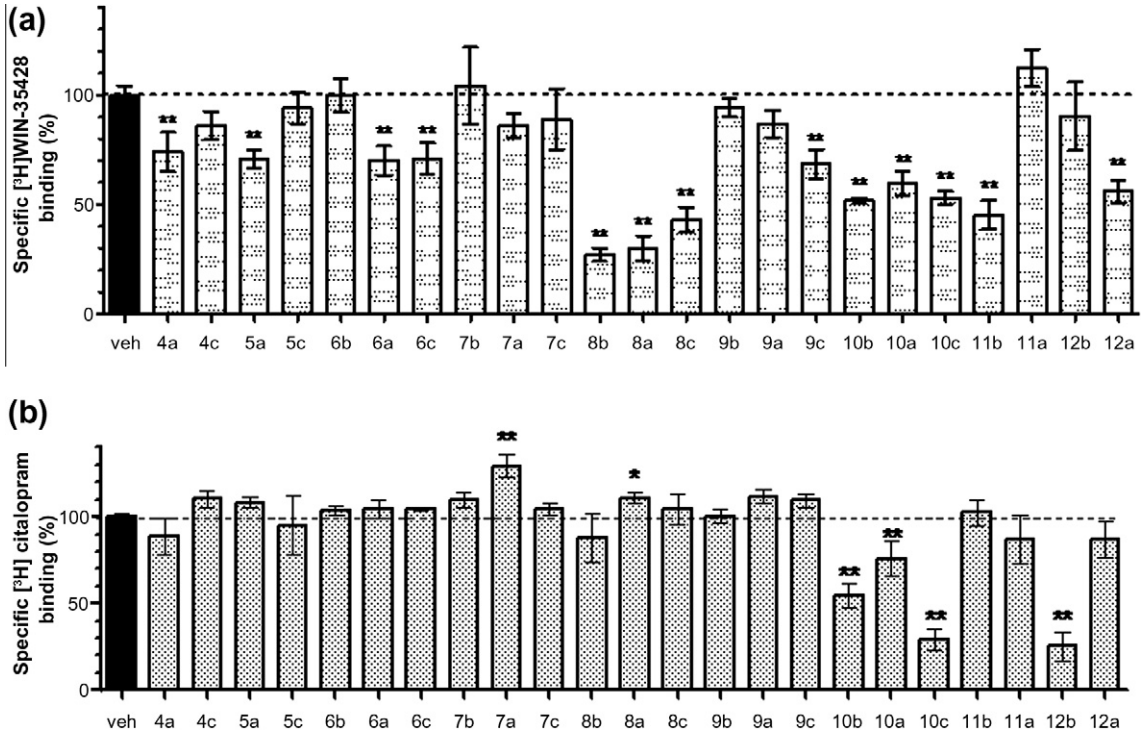



**Figure 2.** (a) Specific binding of [$^3$H]WIN 35,428 (2 nM) to rat striatal dopamine transporter (DAT), in absence (black bar) or presence of 10 μM compounds. Nomifensine (3 μM) was used to define non-specific binding. Each bar represents the mean ± SD of 3–6 replicates obtained from 1 to 2 different experiments. (b) Specific binding of [$^3$H]citalopram (1 nM) to rat brain cortex serotonin transporter (SERT), in absence (black bar) or presence of 10 μM compounds. (*S*)-Citalopram (10 μM) was used to define non-specific binding. Each bar represents the mean ± S.E.M. of 4–16 replicates obtained from 1 to 5 different experiments (**$p$ <0.01, *$p$ <0.05 vs vehicle, Student's $t$ test).

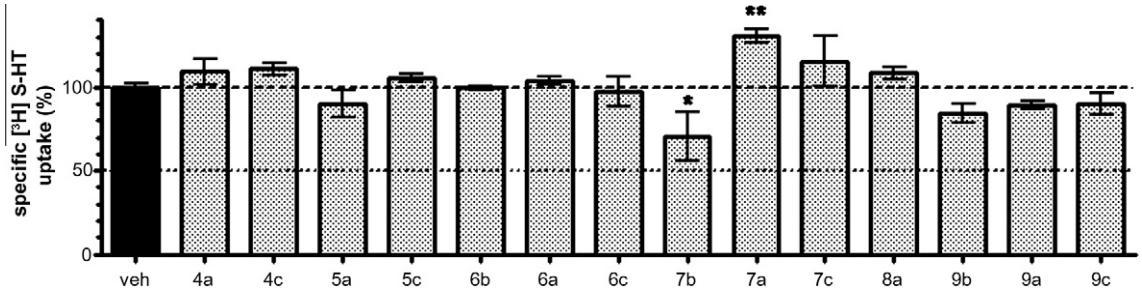



**Figure 3.** Specific uptake of [$^3$H]serotonin (100 nM) in rat brain cortex synaptosomes, in absence (black bar) or presence of 10 μM compounds. (*S*)-Citalopram (10 μM) was used to define non-specific uptake. Each bar represents the mean ± S.E.M. of 4–16 replicates (*$p$ <0.05 vs vehicle, Student's $t$ test).

Finally, we performed saturation experiments to better characterize the effect of **7a** on SERT. Figure 5a shows the saturation curves of [$^3$H]citalopram binding obtained in the absence or presence of 10 μM **7a**. We confirmed the effects of **7a** on binding, with a significant overall effect detected by Two-way's ANOVA ($p$ <0.01), and significant increases detected by Bonferroni's post-test at the two highest [$^3$H]citalopram concentrations ($p$ <0.01). The fitting of the curves highlighted an increase of both $B_{max}$ (+24%) and $K_d$ (+25%), although they did not reach statistical significance ($p$ = 0.06 and $p$ = 0.43, respectively). This finding suggests however that the increase of specific binding is more likely accounted for by an increase of binding sites rather than by an increase of affinity. This suggestion was confirmed when using [$^3$H]paroxetine, another specific ligand for SERT with an even higher affinity (Fig. 5b). Indeed, as reported in Figure 5b, 7**a** (10 μM) induced an increase of binding of this radioligand, which was associated to a noteworthy enhancement of $B_{max}$ (+51%, $p$ <0.05) with no significant change of $K_d$. Figure 5c shows the results on synaptosomal [$^3$H]5-HT uptake. The overall effect of **7a** (10 μM) was small but significant ($p$ <0.01, Two-way's ANOVA). Non-linear fitting of the saturation curves showed higher $V_{max}$ and $K_m$ values in the presence of **7a**, although the differences did not reach statistical significance. As for [$^3$H]citalopram, the data suggest that the small increase of [$^3$H]5-HT uptake is likely due to an increase of $V_{max}$, that is the number of 5-HT molecules taken up per unit of time (proportional to either the number of available SERTs and the rate of SERT-mediated transport).

To the best of our knowledge, **7a** is the first compound which increases the number of [$^3$H]SSRI binding sites in in vitro assays. It had been previously shown, in fact, that tianeptine, whose structure is depicted in Figure 6 together with that of **7a**, increases the $V_{max}$ of synaptosomal [$^3$H]5-HT uptake only after in vivo administration, whereas no effect was observed in vitro.[27] Tianeptine, which shares some structural similarities with tricyclic antidepressants, raised a lot of interest[28] since it shows antidepressant properties despite the fact that its effect on SERT is opposite to that of classical antidepressants, which are inhibitors of the reuptake systems. Although recent studies suggest that the antidepressant

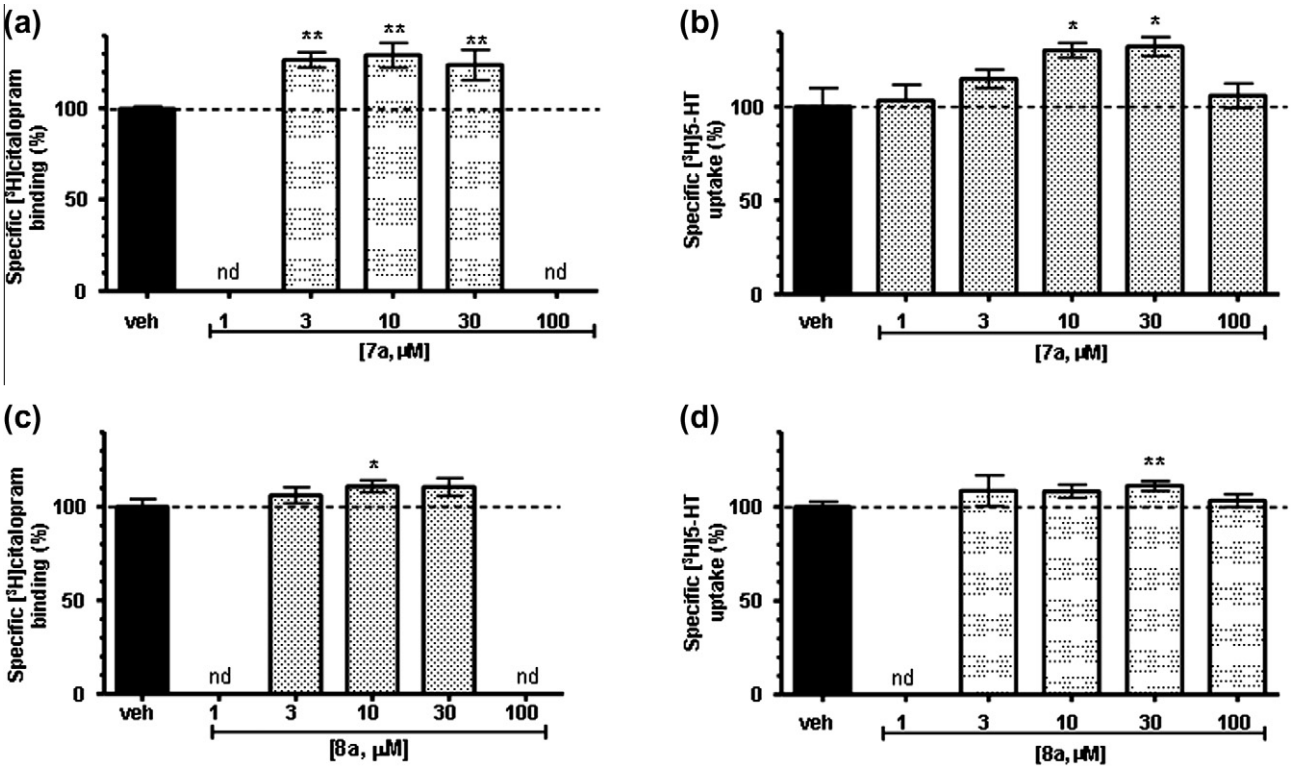
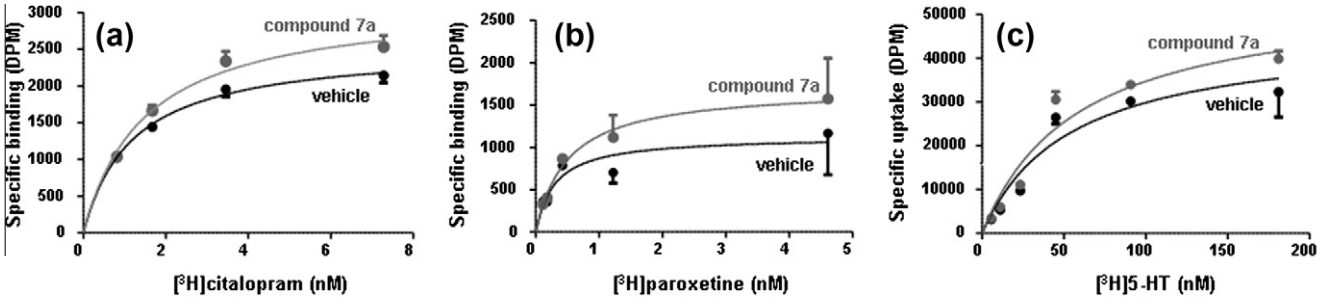



**Figure 4.** Dose-response effect of **7a** on (a) SERT binding, and (b) SERT-mediated uptake, and of **8a** on (c) SERT binding, and (d) SERT-mediated uptake. Specific [$^3$H]citalopram (1 nM) binding was measured in rat brain cortex membranes while specific [$^3$H]serotonin (100 nM) uptake was measured in rat brain cortex synaptosomes. Black bars represent binding and uptake in absence of compounds. (*S*)-Citalopram (10 μM) was used to define non-specific binding and uptake. Each bar represents the mean ± S.E.M. of 3–16 replicates (**$p$ <0.01, *$p$ <0.05 vs vehicle, Student's t test). Nd: not determined.




| | vehicle | 7a |
|---|---|---|
| $B_{max}$ (DPM) | 2538 ± 98 | 3153 ± 223 p=0.06 |
| $K_d$ (nM) | 1.19 ± 0.15 | 1.49 ± 0.31 p=0.43 |

| | vehicle | 7a |
|---|---|---|
| $B_{max}$ (DPM) | 1127 ± 168 | 1703 ± 104 p=0.04 |
| $K_d$ (nM) | 0.30 ± 0.15 | 0.49 ± 0.10 p=0.35 |

| | vehicle | 7a |
|---|---|---|
| $V_{max}$ (DPM) | 45885 ± 5967 | 54916 ± 5462 p=0.24 |
| $K_m$ (nM) | 53.6 ± 16.2 | 58.1 ± 13.9 p=0.81 |

**Figure 5.** Saturation curves of (a) [$^3$H]citalopram binding, (b) [$^3$H]paroxetine binding, and (c) [$^3$H]5-HT uptake, in absence (black) and presence of 10 μM compound **7a**. Radioligand concentration is shown on abscissa while radioactivity (DPM, disintegration per minute) corresponding to specific binding/uptake is shown on ordinates. Each point represents the mean ± SD of 4 replicates. Saturation curves were obtained using the 'one-site binding, hyperbola' equation (GraphPad Prism 4.0a), to obtain $B_{max}/V_{max}$ and $K_d/K_m$, each of them with the corresponding S.E.M. Extra sum-of-squares *F*-test included in the GraphPad Prism software was used to evaluate the statistical significance of the differences due to compound **7a**, and the results of these tests are indicated.

activity of tianeptine is possibly due to SERT-independent mechanisms, with the involvement of the glutamatergic system,[29,30] it could be interesting to investigate if **7a** acts as antidepressant in animal models.

Apart from its therapeutic potential, **7a** might represent a useful tool to investigate the functioning of SERT at a molecular level. The unique effect of the compound, that is the increase of the number of [$^3$H]SSRI binding sites observed in brain membrane fragments, might be explained assuming that **7a** unmasks binding sites which are otherwise not available to the radioligand. Thus, it could be speculated that SERTs might exist in interconverting conformational states and that **7a** could shift the equilibrium from the state not exposing the SSRI binding site to the one exposing it. In this respect, ibogaine, a hallucinogenic drug with putative anti-addiction

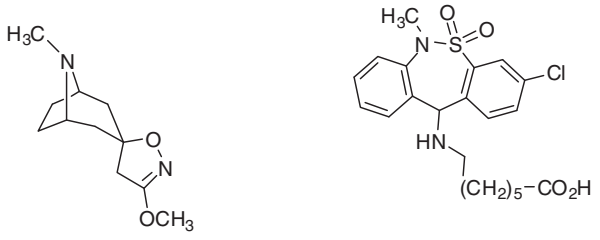



**Figure 6.** Molecular structure of **7a** and tianeptine.

properties, which displayed an opposite profile compared to **7a**, that is a noncompetitive inhibition of SERTs by decreasing $V_{max}$ with little change in the $K_m$ for 5-HT, has been suggested to stabilize the cytoplasm-facing state of the transporter.[31] This behavior of transporter systems is reminiscent of what is known for G-protein coupled receptors, which have been postulated to exist in a conformational equilibrium between active and inactive states, shifted in one direction or the other by agonists or inverse agonists.[32–34] The effect of **7a** on SERT could also be the result of its interaction with a binding site distinct from the one bound by [$^3$H]SSRI or [$^3$H]5-HT, likely an allosteric site, whose existence has been already proposed.[35,36]

## 3. Conclusions

In summary, we designed a group of tertiary/secondary amines and quaternary ammonium salts with the molecular skeleton of spirocyclic tropanyl-$\Delta^2$-isoxazolines. The synthetic approach to these compounds made use of the 1,3-dipolar cycloaddition of nitrile oxides to suitably functionalized olefins. The derivatives did not show relevant affinity for both DAT and SERT but, intriguingly, the 3-methoxy-tropanyl-$\Delta^2$-isoxazoline **7a**, and, to a lesser extent, its 3-ethoxy-substituted analogue **8a**, reproducibly enhanced the binding of specific SERT ligands such as [$^3$H]citalopram and [$^3$H]paroxetine to rat brain cortex membranes. Moreover, **7a** induced a parallel increase of the [$^3$H]5-HT uptake in rat brain cortex synaptosomes. Therefore **7a** represents a novel pharmacological tool potentially useful to deepen our understanding of the functioning of SERT.

## 4. Experimental section

### 4.1. Chemistry

#### 4.1.1. Materials and methods

$^1$H NMR and $^{13}$C NMR spectra were recorded with a Varian Mercury 300 ($^1$H, 300.063; $^{13}$C, 75.451 MHz) spectrometer in $CDCl_3$ solutions (unless otherwise indicated) at 20 °C. TLC analyses were performed on commercial silica gel 60 $F_{254}$ aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or a phosphomolybdic acid solution and, for tertiary amines, with the Dragendorff reagent. Melting points were determined on a model B 540 Büchi apparatus and are uncorrected. ESI mass spectra were obtained on a Varian 320 LC–MS/MS instrument. Data are reported as mass-to-charge ratio ($m/z$) of the corresponding positively charged molecular ions. Microanalyses (C, H, N) agreed with the theoretical value within ±0.4%.

#### 4.1.2. *tert*-Butyl 3-methylene-8-azabicyclo[3.2.1.]octane-8-carboxylate (16)

To an ice-bath cooled and stirred suspension of *tert*-BuOK (1.57 g, 14.0 mmol) in anhydrous THF (60 mL), methyltriphenylphosphonium bromide (5.21 g, 14.58 mmol) was added. After 15 min, the mixture was heated at reflux for 45 min, then cooled at rt, and a solution of *tert*-butyl 3-oxo-8-azabicyclo[3.2.1.]octane-8-carboxylate **15**[25] (2.25 g, 10 mmol) in anhydrous THF (12 mL) was added dropwise. After stirring at rt for 1 h, the reaction was quenched with acetone (10 mL) and the solid was filtered off. The liquid phase was concentrated in vacuo, then water was added (50 mL) and the crude mixture was extracted with ether (5 × 30 mL). The pooled organic phases were dried over anhydrous $Na_2SO_4$, then filtered and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 9:1), which gave alkene **16** (1.87 g, 84% yield).

*Compound **16***: Colorless viscous oil. $R_f$ = 0.52 (cyclohexane/ethyl acetate 4:1) $^1$H NMR $\delta$: 1.48 (s, 9H), 1.55 (m, 2H), 1.86 (m, 2H), 2.07 (d, $J$ = 13.8, 2H), 2.48 (d, $J$ = 13.8, 2H), 4.16 (br s, 1H), 4.27 (br s, 1H), 4.84 (br s, 2H) ppm. $^{13}$C NMR $\delta$: 28.42, 37.95, 39.81, 69.84, 79.88, 107.83, 148.40, 154.42 ppm. $C_{13}H_{21}NO_2$ (223.31): Calcd C, 62.92; H, 9.48; N, 6.27. Found: C, 63.19; H, 9.22; N, 6.03.

#### 4.1.3. *tert*-Butyl 3′-bromo-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-8-carboxylate (17)

To a suspension of alkene **16** (2.24 g, 10.0 mmol) and $NaHCO_3$ (8.40 g, 100 mmol) in ethyl acetate (60 mL) was added dibromoformaldoxime (2.43 g, 12.0 mmol). The reaction mixture was stirred overnight at rt, then Celite® was added and the resulting slurry was filtered under vacuum and washed with ethyl acetate. The solvent was evaporated and the residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 9:1) to afford $\Delta^2$-isoxazoline **17** (2.83 g, 82% yield).

*Compound **17***: Crystallized from *n*-hexane/ethyl acetate (2:1) as a colorless powder, mp 127–128 °C. $R_f$ = 0.43 (cyclohexane/ethyl acetate 4:1). $^1$H NMR $\delta$: 1.47 (s, 9H), 1.70 (m, 2H), 1.93 (d, $J$ = 14.1, 2H), 2.00 (m, 2H), 2.08–2.32 (m, 2H), 3.27 (s, 2H), 4.27 (br s, 1H), 4.37 (br s, 1H) ppm. $^{13}$C NMR $\delta$: 26.91, 27.75, 28.67, 41.33, 42.00, 52.73, 53.34, 55.07, 80.15, 86.39, 136.21, 153.24 ppm. $C_{14}H_{21}BrN_2O_3$ (345.23): Calcd C, 48.71; H, 6.13; N, 8.11. Found: C, 48.94; H, 6.27; N, 7.96.

#### 4.1.4. *tert*-Butyl 3′-methoxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-8-carboxylate (18)

A stirred suspension of bromo-$\Delta^2$-isoxazoline **17** (500 mg, 1.45 mmol) and $K_2CO_3$ (2.0 g, 14.5 mmol) in MeOH (15 mL) was stirred at reflux for 2 h. After addition of Celite® and filtration under vacuum, the crude filtrate was submitted to a silica gel column chromatography (cyclohexane/ethyl acetate 5:1), giving the title compound **18** (387 mg, 90% yield).

*Compound **18***: Crystallized from petroleum ether/ethyl acetate (95:5) as colorless prisms, mp 124.5–125 °C. $R_f$ = 0.36 (cyclohexane/ethyl acetate 7:3). $^1$H NMR $\delta$: 1.47 (s, 9H), 1.65 (m, 2H), 1.94 (m, 4H), 2.02–2.23 (m, 2H), 3.02 (s, 2H), 3.81 (s, 3H), 4.24 (br s, 1H), 4.34 (br s, 1H) ppm. $^{13}$C NMR $\delta$: 26.83, 27.71, 28.64, 41.54, 42.23, 46.85, 52.77, 53.44, 57.23, 79.78, 84.30, 153.29, 166.58 ppm. $C_{15}H_{24}N_2O_4$ (296.36): Calcd C, 60.79; H, 8.16; N, 9.45. Found: C, 60.92; H, 8.39; N, 9.13.

#### 4.1.5. *tert*-Butyl 3′-ethoxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-8-carboxylate (19)

Intermediate **17** (600 mg, 1.74 mmol) and EtOH (15 mL) were reacted following the protocol above reported for the preparation of methoxy-$\Delta^2$-isoxazoline **18**. The crude reaction mixture was submitted to a silica gel column chromatography (cyclohexane/ethyl acetate 5:1), giving the desired title compound **19** (388 mg, 72% yield).

*Compound **19***: Crystallized from petroleum ether/ethyl acetate (9:1) as colorless prisms, mp 111–111.5 °C. $R_f$ = 0.25 (cyclohexane/ethyl acetate 4:1). $^1$H NMR $\delta$: 1.30 (t, $J$ = 7.2, 3H), 1.44 (s,

9H), 1.68 (m, 2H), 1.94 (m, 4H), 1.98–2.20 (m, 2H), 3.00 (s, 2H), 4.14 (q, $J$ = 7.2, 2H), 4.21 (br s, 1H), 4.30 (br s, 1H) ppm. $^{13}$C NMR $\delta$: 14.64, 26.89, 27.70, 28.66, 41.53, 42.19, 46.83, 52.81, 53.42, 65.97, 79.88, 84.19, 153.31, 166.62 ppm. $C_{16}H_{26}N_2O_4$ (310.39): Calcd C, 61.91; H, 8.44; N, 9.03. Found: C, 62.12; H, 8.57; N, 8.84.

#### 4.1.6. *tert*-Butyl 3′-(prop-2-ynyloxy)-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-8-carboxylate (20)

Sodium hydride (197 mg, 8.22 mmol) was added to a stirred solution of propargyl alcohol (408 μL, 7.0 mmol) in anhydrous THF (5 mL) under nitrogen at 0 °C. After 30 min, a solution of **17** (600 mg, 1.74 mmol) in anhydrous THF (5 mL) was added dropwise and the mixture was stirred at reflux for 3 h. After addition of $H_2O$ (1 mL) and concentration in vacuo, the residue was diluted with $H_2O$ (5 mL) and extracted with EtOAc (5 × 5 mL). After the usual workup, the crude reaction mixture was submitted to a silica gel column chromatography (petroleum ether/ethyl acetate 4:1), which provided the desired ether **20** (484 mg, 87% yield).

*Compound* ***20***: Crystallized from petroleum ether/ethyl acetate (9:1) as colorless prisms, mp 102.5–104 °C. $R_f$ = 0.63 (petroleum ether/ethyl acetate 7:3). $^1$H NMR $\delta$: 1.47 (s, 9H), 1.68 (m, 2H), 1.96 (m, 4H), 1.98–2.24 (m, 2H), 2.58 (t, $J$ = 2.2, 1H), 3.08 (s, 2H), 4.24 (br s, 1H), 4.34 (br s, 1H), 4.75 (d, $J$ = 2.2, 2H) ppm. $^{13}$C NMR $\delta$: 26.78, 27.65, 28.65, 41.57, 42.23, 46.79, 52.84, 53.49, 56.32, 79.89, 80.61, 82.37, 84.21, 153.29, 166.64 ppm. $C_{17}H_{24}N_2O_4$ (320.38): Calcd C, 63.73; H, 7.55; N, 8.74. Found: C, 63.79; H, 7.78; N, 8.92.

#### 4.1.7. *tert*-Butyl 3′-benzyloxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-8-carboxylate (21)

Intermediate **17** (2 g, 5.79 mmol) and anhydrous benzyl alcohol (3.6 mL, 34.76 mmol) were reacted following the protocol above reported for the preparation of propargyloxy-$\Delta^2$-isoxazoline **20**. After distillation of excess benzyl alcohol under reduced pressure, the crude reaction mixture was purified by silica gel column chromatography (cyclohexane/ethyl acetate 4:1), giving the desired title compound **21** (1.66 g, 77% yield).

*Compound* ***21***: Crystallized from ethyl acetate as a colorless powder, mp 131.5–132 °C. $R_f$ = 0.51 (cyclohexane/ethyl acetate 7:3). $^1$H NMR $\delta$: 1.47 (s, 9H), 1.67 (m, 2H), 1.98 (m, 4H), 1.98–2.28 (m, 2H), 3.07 (s, 2H), 4.24 (br s, 1H), 4.34 (br s, 1H), 5.12 (s, 2H), 7.36 (s, 5H) ppm. $^{13}$C NMR $\delta$: 26.90, 27.69, 28.70, 41.51, 42.21, 46.78, 52.83, 53.40, 71.95, 79.96, 84.65, 128.62, 128,86, 135.70, 153.35, 166.60 ppm. $C_{21}H_{28}N_2O_4$ (372.46): Calcd C, 67.12; H, 7.58; N, 7.52. Found: C, 66.89; H, 7.67; N, 7.40.

#### 4.1.8. *tert*-Butyl 3′-oxo-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine}-8-carboxylate (22)

A stirred solution of benzyloxy derivative **21** (641 mg, 1.72 mmol) in MeOH (15 mL) was hydrogenated at rt under a balloon pressure in the presence of 10% Pd/C (100 mg) for 6 h. The reaction mixture was filtered and the solvent was evaporated in vacuo to afford the desired isoxazolidin-3-one **22** (462 mg, 95% yield).

*Compound* ***22***: Crystallized from ethyl acetate as colorless prisms, mp 118–119 °C. $R_f$ = 0.33 (cyclohexane/ethyl acetate 2:3). $^1$H NMR $\delta$: 1.41 (s, 9H), 1.61 (m, 2H), 1.85–2.15 (m, 6H), 2.80 (s, 2H), 4.21 (br s, 1H), 4.28 (br s, 1H), 5.26 (br s, 1H) ppm. $^{13}$C NMR $\delta$: 27.08, 27.75, 28.62, 40.42, 41.14, 47.63, 52.37, 53.02, 80.09, 83.66, 153.32, 174.00 ppm. $C_{14}H_{22}N_2O_4$ (282.34): Calcd C, 59.56; H, 7.85; N, 9.92. Found: C, 59.68; H, 8.07; N, 9.83.

#### 4.1.9. *tert*-Butyl 2′-methyl-3′-oxo-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine}-8-carboxylate (23)

Iodomethane (310 μL, 5 mmol) was added to a suspension of isoxazolidin-3-one **22** (200 mg, 0.71 mmol) and $K_2CO_3$ (980 mg, 7.1 mmol) in acetone (10 mL). After stirring at rt for 16 h, the solvent was evaporated, the residue was diluted with $H_2O$ (10 mL) and extracted with EtOAc (3 × 20 mL). After standard workup, the $^1$H NMR spectrum of the crude reaction mixture showed, together with the desired 2′-methyl-3′-oxo-isoxazolidine, the regioisomeric 3′methoxy-substituted-$\Delta^2$-isoxazoline **18** (less than 10%). Purification by silica gel column chromatography (cyclohexane/ethyl acetate 7:3) afforded the desired N-methylated derivative **23** (170 mg, 81% yield).

*Compound* ***23***: Yellow viscous oil, $R_f$ = 0.42 (cyclohexane/ethyl acetate 3:7); $^1$H NMR $\delta$: 1.39 (s, 9H), 1.58 (m, 2H), 1.82–1.97 (m, 4H), 1.95–2.08 (m, 2H), 2.78 (s, 2H), 3.02 (s, 3H), 4.17 (br s, 1H), 4.25 (br s, 1H) ppm. $^{13}$C NMR $\delta$: 27.03, 27.75, 28.61, 31.63, 40.46, 41.23, 47.99, 52.32, 52.99, 79.92, 80.66, 153.18, 168.78 ppm. $C_{15}H_{24}N_2O_4$ (296.36): Calcd C, 60.79; H, 8.16; N, 9.45. Found: C, 60.42; H, 8.31; N, 9.67.

#### 4.1.10. Ethyl 8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-3′-carboxylate (4a)

A solution of ethyl 2-chloro-2-(hydroximino)-acetate (2.88 g, 19.0 mmol) in dioxane (10 mL) was added to a magnetically stirred suspension of 8-methyl-3-methylene-8-azabicyclo[3.2.1]octane **14**[22] (1.30 g, 9.49 mmol) and $NaHCO_3$ (7.97 g, 94.90 mmol) in dioxane (50 mL). The reaction mixture was heated at reflux for 2 days with further addition of aliquots of ethyl 2-chloro-2-(hydroximino)-acetate (2.88 g, 19.0 mmol). After filtration and removal of the solvent at reduced pressure, the residue was purified by silica gel column chromatography (dichloromethane/methanol 9:1) to afford the desired cycloadduct **4a** (311 mg, 13% yield).

*Compound* ***4a***: Pale yellow viscous oil. $R_f$ = 0.69 (dichloromethane/methanol 4:1). $^1$H NMR $\delta$: 1.36 (t, $J$ = 7.0, 3H), 1.74 (m, 4H), 2.06 (m, 2H), 2.29 (dd, $J$ = 3.7 and 13.9, 2H), 2.40 (s, 3H), 3.22 (s, 2H), 3.30 (br s, 2H), 4.33 (q, $J$ = 7.0, 2H) ppm. $^{13}$C NMR $\delta$: 13.81, 26.94, 27.15, 39.54, 41.12, 62.17, 65.48, 82.88, 151.29, 163.41 ppm. $C_{13}H_{20}N_2O_3$ (252.31): Calcd C, 61.88; H, 7.99; N, 11.10. Found: C, 62.20; H, 7.63; N, 10.88.

#### 4.1.11. 3′-Hydroxymethyl-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (5a)

Sodium borohydride (450 mg, 11.88 mmol) was added portionwise to a magnetically stirred solution of ester **4a** (1 g, 3.96 mmol) in EtOH (20 mL) at rt After 3 h, 5 mL of 2 N HCl were added (pH about 3), the solvent was evaporated and the acidic residue was extracted with ethyl acetate (3 × 5 mL). The residual aqueous phase, made basic by portionwise addition of solid $K_2CO_3$ (pH 10), was repeatedly extracted with dichloromethane (15 × 5 mL). The pooled organic phases were dried over anhydrous $Na_2SO_4$, then filtered and concentrated in vacuo to provide the primary alcohol **5a** (316 mg, 38% yield).

*Compound* ***5a***: Yellow gummy derivative. $R_f$ = 0.15 (dichloromethane/methanol 7:3). $^1$H NMR $\delta$: 1.70 (m, 5H), 2.04 (m, 2H), 2.23 (d, $J$ = 3.6 and 14.0, 2H), 2.38 (s, 3H), 3.06 (s, 2H), 3.26 (m, 2H), 4.38 (s, 2H) ppm. $^{13}$C NMR $\delta$: 27.07, 33.54, 39.51, 41.44, 65.49, 69.03, 83.45, 164.68 ppm. $C_{11}H_{18}N_2O_2$ (210.27): Calcd C, 62.83; H, 8.63; N, 13.32. Found: C, 62.49; H, 8.90; N, 13.02.

#### 4.1.12. Synthesis of secondary amines 6b–10b

To an ice-bath cooled and stirred solution of *N*-Boc protected derivative (1.5 mmol) in dichloromethane (10 mL), a solution of trifluoracetic acid (1.16 mL, 15 mmol) in $CH_2Cl_2$ (5.7 mL) was added dropwise, and the mixture was stirred at rt until disappearance of the starting material (from 3 to 18 h, TLC monitoring). After concentration at reduced pressure, the residue was dissolved in $H_2O$ (15 mL) and treated with ether (3 × 10 mL). The residual aqueous phase was made alkaline by portionwise addition of solid $K_2CO_3$ (pH 10) and extracted with dichloromethane (5 × 10 mL).

The combined organic phases were dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure providing the required secondary base.

**4.1.12.1. 3′-Bromo-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (6b).** Eighty-nine percentage yield, crystallized from ethyl acetate as colorless prisms, mp 109–110 °C, dec $R_f$ = 0.48 (dichloromethane/methanol 9:1). $^1$H NMR $\delta$: 1.66 (m, 2H), 1.82 (m, 2H), 1.92 (dd, $J$ = 2.2 and 14.0, 2H), 2.00 (dd, $J$ = 3.6 and 13.7, 2H), 2.12 (br s, 1H), 3.17 (s, 2H), 3.62 (m, 2H) ppm. $^{13}$C NMR $\delta$: 27.52, 28.13, 28.32, 42.79, 44.01, 54.34, 54.53, 86.57, 136.34 ppm. $C_9H_{13}BrN_2O$ (245.12): Calcd C, 44.10; H, 5.35; N, 11.43. Found: C, 44.32; H, 5.11; N, 11.50.

**4.1.12.2. 3′-Methoxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (7b).** Eighty-seven percentage yield, crystallized from ether/acetone (7:3) as light yellow prisms, mp 113–114 °C, dec $R_f$ = 0.47 (dichloromethane/methanol 4:1). $^1$H NMR $\delta$: 1.73 (m, 2H), 1.98 (m, 2H), 2.08 (m, 2H), 2.15 (m, 2H), 2.88 (br s, 1H), 2.99 (s, 2H), 3.77 (m, 2H), 3.84 (s, 3H) ppm. $^{13}$C NMR $\delta$: 27.84, 44.12, 46.17, 54.05, 57.88, 84.20, 166.22 ppm. $C_{10}H_{16}N_2O_2$ (196.25): Calcd C, 61.20; H, 8.22; N, 14.27. Found: C, 61.35; H, 8.10; N, 14.21.

**4.1.12.3. 3′-Ethoxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (8b).** Eighty-three percentage yield, pale yellow viscous oil. $R_f$ = 0.26 (dichloromethane/methanol 4:1). $^1$H NMR $\delta$: 1.33 (t, $J$ = 7.0, 3H), 1.71 (m, 4H, 1H exchanges with $D_2O$), 1.86 (m, 2H), 2.00 (m, 3H), 2.98 (s, 2H), 3.67 (m, 2H), 4.16 (q, $J$ = 7.0, 2H) ppm. $^{13}$C NMR $\delta$: 14.52, 27.97, 44.09, 46.14, 54.24, 65.67, 84.20, 166.54 ppm. $C_{11}H_{18}N_2O_2$ (210.27): Calcd C, 62.83; H, 8.63; N, 13.32. Found: C, 63.19; H, 8.38; N, 13.11.

**4.1.12.4. 3′-(Prop-2-ynyloxy)-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (9b).** Eighty-five percentage yield, yellow viscous oil. $R_f$ = 0.81 (dichloromethane/methanol 4:1). $^1$H NMR $\delta$: 1.69 (m, 2H), 1.84 (m, 2H), 1.92 (br s, 1H, exchanges with $D_2O$), 1.99 (m, 4H), 2.57 (t, $J$ = 1.8, 1H), 3.02 (s, 2H), 3.66 (m, 2H), 4.72 (d, $J$ = 1.8, 2H) ppm. $^{13}$C NMR $\delta$: 28.02, 44.21, 46.30, 54.19, 57.12, 79.04, 81.67, 84.32, 167.20 ppm. $C_{12}H_{16}N_2O_2$ (220.27): Calcd C, 65.43; H, 7.32; N, 12.72. Found: C, 65.07; H, 7.58; N, 12.96.

**4.1.12.5. 3′-Benzyloxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (10b).** Fifty-eight percentage yield, yellow viscous oil. $R_f$ = 0.27 (cyclohexane/ethyl acetate 2:3). $^1$H NMR $\delta$: 1.68 (m, 3H, 1H exchanges with $D_2O$), 1.83 (m, 2H), 2.01 (m, 4H), 3.03 (s, 2H), 3.65 (m, 2H), 5.12 (s, 2H), 7.38 (s, 5H) ppm. $^{13}$C NMR $\delta$: 28.14, 44.41, 46.26, 54.41, 71.86, 84.87, 128.59, 128.80, 128.85, 135.78, 166.75 ppm. $C_{16}H_{20}N_2O_2$ (272.34): Calcd C, 70.56; H, 7.40; N, 10.29. Found: C, 70.82; H, 7.18; N, 10.13.

### 4.1.13. Synthesis of tertiary amines 6a–12a

A 37% aqueous solution of formaldehyde (373 μL, 5 mmol) was added to a solution of secondary amine (1 mmol) in MeOH (5 mL). The reaction mixture was stirred at rt (from 15 min to 2 h), then was cooled at 0 °C, and $NaBH_4$ (95 mg, 2.5 mmol) was added portionwise. After about 1 h, further $NaBH_4$ (95 mg, 2.5 mmol) was added to the reaction, which was stirred until disappearance of the intermediate Schiff base (from 2 to 8 h, TLC monitoring). After concentration at reduced pressure, the residue was partitioned between ethyl ether and acidic water (pH 2). The residual aqueous phase (about 10 mL), after addition of solid $K_2CO_3$ (pH 10), was extracted with dichloromethane (5 × 10 mL). After the usual workup, the crude reaction mixture was column chromatographed to give the wanted tertiary amine.

**4.1.13.1. 3′-Bromo-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (6a).** Fifty-seven percentage yield, crystallized from ethyl acetate as a colorless powder, mp 127–128.5 °C. $R_f$ = 0.49 (dichloromethane/methanol 9:1). $^1$H NMR $\delta$: 1.59 (m, 2H), 1.77 (dd, $J$ = 1.9 and 15.1, 2H), 2.02 (m, 2H), 2.21 (dd, $J$ = 3.6 and 14.0, 2H), 2.32 (s, 3H), 3.18 (s, 2H), 3.23 (m, 2H) ppm. $^{13}$C NMR $\delta$: 25.61, 26.00, 27.53, 38.12, 40.35, 40.46, 60.06, 60.25, 86.28, 136.20 ppm. $C_{10}H_{15}BrN_2O$ (259.14): Calcd C, 46.35; H, 5.83; N, 10.81. Found: C, 46.09; H, 6.07; N, 10.58.

**4.1.13.2. 3′-Methoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′H)-isoxazole} (7a).** Forty-seven percentage yield, light yellow thick oil. $R_f$ = 0.48 (dichloromethane/methanol 4:1). $^1$H NMR $\delta$: 1.62 (m, 2H), 1.82 (m, 2H), 2.03 (m, 2H), 2.20 (m, 2H), 2.37 (s, 3H), 2.96 (s, 2H), 3.25 (m, 2H), 3.82 (s, 3H) ppm. $^{13}$C NMR: 27.78, 40.23, 44.15, 46.22, 54.45, 57.92, 84.37, 166.35 ppm. $C_{11}H_{18}N_2O_2$ (210.27): Calcd C, 62.83; H, 8.63; N, 13.32. Found: C, 62.51; H, 8.84; N, 13.60.

**4.1.13.3. 3′-Ethoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (8a).** Eighty-three percentage yield, colorless thick oil. $R_f$ = 0.28 (dichloromethane/methanol 9:1). $^1$H NMR $\delta$: 1.23 (t, $J$ = 7.1, 3H), 1.54 (m, 2H), 1.74 (dd, $J$ = 1.9 and 13.3, 2H), 1.93 (m, 2H), 2.08 (dd, $J$ = 3.6 and 13.8, 2H), 2.26 (s, 3H), 2.88 (s, 2H), 3.14 (m, 2H), 4.06 (q, $J$ = 7.1, 2H) ppm. $^{13}$C NMR $\delta$: 14.58, 25.85, 38.17, 40.58, 46.93, 60.11, 65.72, 83.92, 166.61 ppm. $C_{12}H_{20}N_2O_2$ (224.30): Calcd C, 64.26; H, 8.99; N, 12.49. Found: C, 64.48; H, 8.73; N, 12.65.

**4.1.13.4. 3′-(Prop-2-ynyloxy)-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (9a).** Seventy-one percentage yield, light yellow thick oil. $R_f$ = 0.46 (dichloromethane/methanol 4:1). $^1$H NMR $\delta$: 1.62 (m, 2H), 1.82 (m, 2H), 2.02 (m, 2H), 2.13 (d, $J$ = 2.6, 1H), 2.16 (d, $J$ = 2.6, 1H), 2.35 (s, 3H), 2.55 (s, 1H), 3.00 (s, 2H), 3.24 (br s, 2H), 4.72 (s, 2H) ppm. $^{13}$C NMR $\delta$: 27.95, 39.98, 44.32, 46.11, 54.38, 57.23, 79.17, 81.65, 84.29, 167.33 ppm. $C_{13}H_{18}N_2O_2$ (234.29): Calcd C, 66.64; H, 7.74; N, 11.96. Found: C, 66.88; H, 7.53; N, 11.71.

**4.1.13.5. 3′-Benzyloxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} (10a).** Ninety percentage yield, yellow viscous oil. $R_f$ = 0.24 (dichloromethane/methanol 9:1). $^1$H NMR $\delta$: 1.64 (m, 2H), 1.86 (dd, $J$ = 1.4 and 14.3, 2H), 2.02 (m, 2H), 2.22 (dd, $J$ = 3.3 and 13.6, 2H), 2.37 (s, 3H), 3.02 (s, 2H), 3.26 (m, 2H), 5.12 (s, 2H), 7.38 (s, 5H) ppm. $^{13}$C NMR $\delta$: $\delta$: 25.95, 38.11, 40.50, 46.96, 60.15, 71.83, 84.42, 128.61, 128.78, 128.84, 135.81, 166.71 ppm. $C_{17}H_{22}N_2O_2$ (286.37): Calcd C, 71.30; H, 7.74; N, 9.78. Found: C, 70.94; H, 7.56; N, 10.09.

**4.1.13.6. 3′-Oxo-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine} (11a).** The tertiary base was obtained in 91% yield by means of catalytic hydrogenation as reported for the synthesis of **22**.

*Compound **11a***: Crystallized from ethyl acetate as colorless prisms, mp 140–145 °C. $R_f$ = 0.20 (dichloromethane/methanol 7:3). $^1$H NMR $\delta$: 1.30 (m, 2H), 1.44 (m, 2H), 2.07 (m, 2H), 2.14 (s, 3H), 2.24 (s, 2H), 2.27 (m, 2H), 3.16 (m, 2H), 5.91 (s, 1H, exchanges with $D_2O$) ppm. $^{13}$C NMR $\delta$: 28.71, 40.15, 44.95, 48.49, 58.86, 67.95, 174.21 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{10}H_{16}N_2O_2$: 196.3. Found: 197.0. $C_{10}H_{16}N_2O_2$ (196.25): Calcd C, 61.20; H, 8.22; N, 14.27. Found: C, 61.37; H, 8.41; N, 14.02.

**4.1.13.7. 2′-Methyl-3′-oxo-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine} (12a).** This compound was obtained in 70% yield by reaction of secondary amine **12b**, in turn generated from hydrochloride **12b** × **HCl** (see below).

*Compound* ***12a***: Crystallized from *n*-hexane/ethyl acetate (7:3) as light yellow prisms, mp 192.5–194 °C. $R_f$ = 0.35 (dichloromethane/methanol 9:1). $^1$H NMR $\delta$: 1.56 (m, 2H), 1.83 (d, $J$ = 12.7, 2H), 2.00 (m, 2H), 2.08 (dd, $J$ = 3.3 and 13.6, 2H), 2.31 (s, 3H), 2.77 (s, 2H), 3.07 (s, 3H), 3.21 (m, 2H) ppm. $^{13}$C NMR $\delta$: 25.89, 31.64, 38.39, 39.89, 48.34, 59.92, 80.57, 169.06 ppm. $C_{11}H_{18}N_2O_2$ (210.27): Calcd C, 62.83; H, 8.63; N, 13.32. Found: C, 63.09; H, 8.78; N, 13.10.

### 4.1.14. Preparation of fumarates of 4a–10a and 6b–10b

A solution of fumaric acid (102 mg, 0.88 mmol) in MeOH (2 mL) was added to a solution of the free base (0.8 mmol) in MeOH (3 mL). After stirring overnight at rt, the solvent was removed at reduced pressure and the crude fumarate, which was obtained quantitatively, was purified by crystallization.

**4.1.14.1. Ethyl 8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-3′-carboxylate fumarate (4a × $C_4H_4O_4$).** Crystallized from abs. ethanol as colorless prisms, mp 220–224 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 1.33 (t, $J$ = 7.3, 3H), 2.17–2.35 (m, 6H), 2.41 (dd, $J$ = 2.7 and 15.0, 2H), 2.81 (s, 3H), 3.48 (s, 2H), 3.97 (br s, 2H), 4.31 (q, $J$ = 7.3, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 15.65, 22.88, 38.57, 40.23, 45.17, 63.57, 67.21, 80.92, 134.87, 167.32, 170.34 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{13}H_{20}N_2O_3$: 252.3. Found: 253.1. $C_{17}H_{24}N_2O_7$ (368.38): Calcd C, 55.43; H, 6.57; N, 7.60. Found: C, 55.66; H, 6.71; N, 7.42.

**4.1.14.2. 3′-Hydroxymethyl-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (5a × $C_4H_4O_4$).** Crystallized from methanol as colorless prisms, mp 223–227.5 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.16–2.38 (m, 8H), 2.80 (s, 3H), 3.27 (s, 2H), 3.95 (br s, 2H), 4.28 (s, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.66, 38.18, 40.06, 45.11, 63.41, 69.71, 80.75, 134.92, 167.29, 170.18 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{11}H_{18}N_2O_2$: 210.3. Found: 211.2. $C_{15}H_{22}N_2O_6$ (326.35): Calcd C, 55.21; H, 6.79; N, 8.58. Found: C, 55.38; H, 6.53; N, 8.71.

**4.1.14.3. 3′-Bromo-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (6a × $C_4H_4O_4$).** Crystallized from 2-propanol/methanol (9:1) as colorless prisms, mp 180–183.5 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.18 (m, 2H), 2.24–2.35 (m, 4H), 2.40 (dd, $J$ = 3.3 and 15.0, 2H), 2.80 (s, 3H), 3.55 (s, 2H), 3.95 (br s, 2H), 6.69 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.41, 38.50, 40.11, 45.37, 63.39, 80.62, 135.02, 167.14, 170.21 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{10}H_{15}BrN_2O$: 259.1. Found: 260.0. $C_{14}H_{19}BrN_2O_5$ (375.22): Calcd C, 44.81; H, 5.10; N, 7.47. Found: C, 44.88; H, 5.29; N, 7.30.

**4.1.14.4. 3′-Bromo-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (6b × $C_4H_4O_4$).** Crystallized from abs. ethanol/methanol (7:3) as colorless prisms, mp 185–187 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.12 (m, 5H), 2.27 (m, 3H), 3.53 (s, 2H), 4.13 (br s, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.31, 38.27, 45.19, 63.42, 80.64, 135.11, 167.26, 170.45 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_9H_{13}BrN_2O$: 245.1. Found: 246.1. $C_{13}H_{17}BrN_2O_5$ (361.19): Calcd C, 43.23; H, 4.74; N, 7.76. Found: C, 43.52; H, 4.76; N, 7.62.

**4.1.14.5. 3′-Methoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (7a × $C_4H_4O_4$).** Crystallized from 2-propanol/methanol (3:7) as colorless prisms, mp 207–226 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.15 (m, 2H), 2.32 (m, 6H), 2.79 (s, 3H), 3.31 (m, 2H), 3.82 (s, 3H), 3.95 (br s, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.45, 38.15, 40.21, 45.30, 57.13, 63.25, 80.43, 134.99, 167.17, 170.15 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{11}H_{18}N_2O_2$: 210.3. Found: 211.1. $C_{15}H_{22}N_2O_6$ (326.35): Calcd C, 55.21; H, 6.79; N, 8.58. Found: C, 55.09; H, 6.85; N, 8.42.

**4.1.14.6. 3′-Methoxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (7b × $C_4H_4O_4$).** Crystallized from 2-propanol/methanol (4:1) as colorless prisms, mp 197–217 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.11 (m, 4H), 2.19 (dd, $J$ = 3.7 and 15.4, 2H), 2.28 (d, $J$ = 13.2, 2H), 3.22 (s, 2H), 3.82 (s, 3H), 4.12 (br s, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.39, 38.21, 45.27, 57.41, 63.57, 80.32, 135.10, 167.24, 170.36 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{10}H_{16}N_2O_2$: 196.3. Found: 197.2. $C_{14}H_{20}N_2O_6$ (312.32): Calcd C, 53.84; H, 6.45; N, 8.97. Found: C, 54.06; H, 6.71; N, 9.12.

**4.1.14.7. 3′-Ethoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (8a × $C_4H_4O_4$).** Crystallized from 2-propanol as colorless prisms, mp 214–216 °C. $^1$H NMR ($CD_3OD$) $\delta$: 1.33 (t, $J$ = 7.1, 3H), 2.15 (m, 2H), 2.31 (m, 6H), 2.80 (s, 3H), 3.25 (s, 2H), 3.95 (br s, 2H), 4.13 (q, $J$ = 7.1, 2H), 6.69 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 13.49, 22.77, 38.37, 40.16, 45.03, 63.22, 66.03, 80.87, 135.05, 167.20, 170.23 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{12}H_{20}N_2O_2$: 224.3. Found: 225.1. $C_{16}H_{24}N_2O_6$ (340.37): Calcd C, 56.46; H, 7.11; N, 8.23. Found: C, 56.60; H, 6.97; N, 8.39.

**4.1.14.8. 3′-Ethoxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (8b × $C_4H_4O_4$).** Crystallized from 2-propanol as colorless prisms, mp 232–233 °C. $^1$H NMR ($CD_3OD$) $\delta$: 1.33 (t, $J$ = 7.1, 3H), 2.11 (m, 4H), 2.20 (dd, $J$ = 3.3 and 14.8, 2H), 2.28 (d, $J$ = 15.1, 2H), 3.21 (s, 2H), 4.12 (br s, 2H), 4.14 (q, $J$ = 7.1, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 13.45, 24.64, 39.47, 44.78, 55.15, 66.00, 81.52, 134.96, 167.20, 169.97 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{11}H_{18}N_2O_2$: 210.3. Found: 211.1. $C_{15}H_{22}N_2O_6$ (326.35): Calcd C, 55.21; H, 6.79; N, 8.58. Found: C, 55.47; H, 6.61; N, 8.73.

**4.1.14.9. 3′-(Prop-2-ynyloxy)-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (9a × $C_4H_4O_4$).** Crystallized from methanol/2-propanol (drops) as pale yellow prisms, mp 165–182 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.19 (m, 2H), 2.33 (m, 6H), 2.80 (s, 3H), 3.05 (s, 1H), 3.32 (m, 2H), 3.95 (br s, 2H), 4.74 (s, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.82, 38.15, 40.34, 45.67, 56.44, 63.36, 79.93, 80.69, 80.75, 135.22, 167.19, 170.38 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{13}H_{18}N_2O_2$: 234.3, found: 235.1. $C_{17}H_{22}N_2O_6$ (350.37): Calcd C, 58.28; H, 6.33; N, 8.00. Found: C, 58.39; H, 6.50; N, 7.86.

**4.1.14.10. 3′-(Prop-2-ynyloxy)-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (9b × $C_4H_4O_4$).** Crystallized from 2-propanol/methanol (9:1) as colorless prisms, mp 160–172 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.11 (m, 4H), 2.19 (dd, $J$ = 2.9 and 14.8, 2H), 2.29 (d, $J$ = 13.2, 2H), 3.07 (t, $J$ = 2.2, 1H), 3.26 (s, 2H), 4.12 (br s, 2H), 4.74 (d, $J$ = 2.2, 2H), 6.68 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.75, 38.23, 45.61, 56.78, 63.12, 79.85, 80.55, 80.92, 135.12, 167.25, 170.16 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{12}H_{16}N_2O_2$: 220.3. Found: 221.1. $C_{16}H_{20}N_2O_6$ (336.34): Calcd C, 57.14; H, 5.99; N, 8.33. Found: C, 57.37; H, 6.12; N, 8.14.

**4.1.14.11. 3′-Benzyloxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (10a × $C_4H_4O_4$).** Crystallized from 2-propanol as colorless prisms, mp 210–211.5 °C. $^1$H NMR ($CD_3OD$) $\delta$: 2.08–2.24 (m, 4H), 2.26–2.37 (m, 4H), 2.80 (s, 3H), 3.29 (s, 2H), 3.95 (br s, 2H), 5.11 (s, 2H), 6.69 (s, 2H), 7.38 (m, 5H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.76, 39.50, 40.16, 44.99, 55.16, 71.75, 81.15, 128.15, 128.39, 135.03, 135.79, 167.06, 170.11 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{17}H_{22}N_2O_2$: 286.4. Found: 287.2. $C_{21}H_{26}N_2O_6$ (402.44): Calcd C, 62.67; H, 6.51; N, 6.96. Found: C, 62.51; H, 6.68; N, 7.11.

**4.1.14.12. 3′-Benzyloxy-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} fumarate (10b × $C_4H_4O_4$).** Crystallized from 2-propanol as colorless prisms, mp 206–208.5 °C. $^1$H NMR ($CD_3OD$) $\delta$: 2.11 (m, 4H), 2.21 (dd, $J$ = 3.3 and 15.1, 2H), 2.30 (d, $J$ = 12.9, 2H), 3.28 (s, 2H), 4.13 (br s, 2H), 5.12 (s, 2H), 6.68 (s, 2H), 7.37 (m, 5H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.63, 39.51, 44.70, 55.17, 71.72, 81.81, 128.15, 128.39, 135.04, 135.80, 167.06, 170.29 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{16}H_{20}N_2O_2$: 272.3. Found: 273.3. $C_{20}H_{24}N_2O_6$ (388.41): Calcd C, 61.84; H, 6.23; N, 7.21. Found: C, 61.93; H, 6.30; N, 7.04.

### 4.1.15. Preparation of iodomethylates 4c–10c

Iodomethane (310 μL, 5 mmol) was added to a solution of the free tertiary amine (0.5 mmol) in MeOH (3 mL). The solution was left overnight at rt, then the solvent was removed at reduced pressure affording quantitatively the crude quaternary salt, which was purified by crystallization.

**4.1.15.1. Ethyl 8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole}-3′-carboxylate methyl iodide (4c).** Crystallized from ethanol/2-propanol (4:1) as yellow prisms, mp 171–178 °C, dec. $^1$H NMR ($D_2O$) $\delta$: 1.43 (t, $J$ = 7.0, 3H), 2.07 (m, 2H), 2.17 (d, $J$ = 15.2, 2H), 2.30 (m, 2H), 2.51 (d, $J$ = 3.7 and 11.0, 2H), 2.96 (s, 3H), 3.11 (s, 3H), 3.41 (s, 2H), 3.83 (br s, 2H), 4.16 (q, $J$ = 7.0, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: $\delta$: 15.03, 23.75, 38.59, 44.02, 45.17, 50.10, 63.14, 71.58, 79.85, 167.32, 170.12 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{14}H_{23}N_2O_3^+$: 267.3. Found: 267.1. $C_{14}H_{23}IN_2O_3$ (394.25): Calcd C, 42.65; H, 5.88; N, 7.11. Found: C, 42.57; H, 6.06; N, 7.32.

**4.1.15.2. 3′-Hydroxymethyl-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} methyl iodide (5c).** Crystallized from methanol as colorless prisms, mp 249–253 °C, dec. $^1$H NMR ($D_2O$) $\delta$: 2.08 (m, 4H), 2.28 (m, 2H), 2.44 (dd, $J$ = 3.7 and 16.5, 2H), 2.94 (s, 3H), 3.08 (s, 3H), 3.23 (s, 2H), 3.80 (br s, 2H), 4.18 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.17, 38.61, 43.76, 45.32, 49.88, 69.65, 71.46, 79.55, 166.92 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{12}H_{21}N_2O_2^+$: 225.3. Found: 225.1. $C_{12}H_{21}IN_2O_2$ (352.21): Calcd C, 40.92; H, 6.01; N, 7.95. Found: C, 41.15; H, 5.85; N, 7.84.

**4.1.15.3. 3′-Bromo-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} methyl iodide (6c).** Crystallized from abs. Ethanol as pale yellow prisms, mp 180–189 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.25 (m, 2H), 2.37 (m, 2H), 2.47 (m, 2H), 2.65 (dd, $J$ = 3.7 and 16.5, 1H), 3.16 (s, 3H), 3.29 (s, 3H), 3.63 (s, 2H), 3.99 (br s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.32, 38.49, 43.55, 45.28, 49.74, 71.22, 79.60, 167.03 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{11}H_{18}BrN_2O^+$: 274.2. Found: 273.9. $C_{11}H_{18}BrIN_2O$ (401.08): Calcd C, 32.94; H, 4.52; N, 6.98. Found: C, 33.18; H, 4.66; N, 6.77.

**4.1.15.4. 3′-Methoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} methyl iodide (7c).** Crystallized from 2-propanol/methanol (9:1) as pale yellow prisms, mp 186–189 °C. $^1$H NMR ($D_2O$) $\delta$: 2.01 (m, 2H), 2.22 (m, 4H), 2.39 (d, $J$ = 3.3, 1H), 2.44 (d, $J$ = 3.3, 1H), 2.97 (s, 3H), 3.10 (s, 3H), 3.19 (s, 2H), 3.67 (s, 3H), 3.82 (br s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.17, 38.36, 43.40, 45.39, 49.88, 57.32, 71.58, 79.39, 167.22 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{12}H_{21}N_2O_2^+$: 225.3. Found: 225.2. $C_{12}H_{21}IN_2O_2$ (352.21): Calcd C, 40.92; H, 6.01; N, 7.95. Found: C, 40.83; H, 6.17; N, 8.09.

**4.1.15.5. 3′-Ethoxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} methyl iodide (8c).** Crystallized from 2-propanol/methanol (9:1) as colorless prisms, mp 213.5–214.5 °C. $^1$H NMR ($CD_3OD$) $\delta$: 1.34 (t, $J$ = 7.1, 3H), 2.23 (m, 2H), 2.37 (d, $J$ = 16.0, 2H), 2.46 (m, 2H), 2.57 (dd, $J$ = 3.6 and 16.2, 2H), 3.15 (s, 3H), 3.26 (s, 3H), 3.30 (s, 2H), 3.97 (s, 2H), 4.15 (q, $J$ = 7.1, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 13.48, 23.82, 38.19, 43.85, 49.98, 63.44, 66.14, 69.02, 79.10, 167.40 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{13}H_{23}N_2O_2^+$: 239.3. Found: 239.1. $C_{13}H_{23}IN_2O_2$ (366.24): Calcd C, 42.63; H, 6.33; N, 7.65. Found: C, 42.85; H, 6.47; N, 7.48.

**4.1.15.6. 3′-(Prop-2-ynyloxy)-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} methyl iodide (9c).** Crystallized from diisopropil ether/methanol (1:1) as light yellow prisms, mp 179–189 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.22 (m, 2H), 2.45 (m, 4H), 2.56 (d, $J$ = 3.3, 1H), 2.62 (d, $J$ = 3.3, 1H), 3.06 (s, 1H), 3.15 (s, 3H), 3.26 (s, 3H), 3.34 (s, 2H), 3.97 (br s, 2H), 4.75 (s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.75, 38.52, 43.11, 45.31, 49.76, 56.80, 71.67, 79.49, 79.84, 80.57, 167.14 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{14}H_{21}N_2O_2^+$: 249.3. Found: 249.1. $C_{14}H_{21}IN_2O_2$ (376.23): Calcd C, 44.69; H, 5.63; N, 7.45. Found: C, 44.47; H, 5.80; N, 7.59.

**4.1.15.7. 3′-Benzyloxy-8-methyl-spiro{8-azabicyclo[3.2.1]octane-3,5′(4′*H*)-isoxazole} methyl iodide (10c).** Crystallized from 2-propanol as colorless powder, mp 244–245 °C. $^1$H NMR ($CD_3OD$) $\delta$: 2.24 (m, 2H), 2.38 (d, $J$ = 17.6, 2H), 2.46 (m, 2H), 2.57 (dd, $J$ = 3.4 and 16.4, 2H), 3.15 (s, 3H), 3.27 (s, 3H), 3.36 (s, 2H), 3.98 (br s, 2H), 5.13 (s, 2H), 7.37 (m, 5H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 23.86, 38.22, 43.93, 45.12, 49.97, 69.02, 71.78, 79.48, 128.20, 128.44, 135.69, 167.35 ppm. MS (ESI) $m/z$ $[M]^+$ Calcd for $C_{18}H_{25}N_2O_2^+$: 301.4. Found: 301.2. $C_{18}H_{25}IN_2O_2$ (428.31): Calcd C, 50.48; H, 5.88; N, 6.54. Found: C, 50.39; H, 5.62; N, 6.71.

### 4.1.16. Preparation of hydrochlorides of 11b, 12a, and 12b

**4.1.16.1. 3′-Oxo-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine} hydrochloride (11b × HCl).** The salt was obtained in 75% yield by treating a solution of **22** (130 mg, 0.46 mmol) in dioxane (1 mL) with a 4 N solution of HCl in dioxane (1 mL) at 0 °C for 2 h.

*Compound **11b** × **HCl***: Crystallized from 2-propanol as colorless prisms, mp 243–248.5 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.11 (m, 4H), 2.21 (dd, $J$ = 3.6 and 15.0, 2H), 2.37 (d, $J$ = 14.3, 2H), 2.98 (s, 2H), 4.16 (br s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.68, 38.56, 45.63, 54.96, 80.49, 173.34 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_9H_{14}N_2O_2$: 182.2. Found: 183.1. $C_9H_{15}ClN_2O_2$ (218.68): Calcd C, 49.43; H, 6.91; Cl, 16.21; N, 12.81. Found: C, 49.48; H, 7.08; Cl, 16.42; N, 12.63.

**4.1.16.2. 2′-Methyl-3′-oxo-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine} hydrochloride (12a × HCl).** The salt was obtained quantitatively by treating a solution of **12a** (105 mg, 0.50 mmol) in $Et_2O$ (2 mL) with a 2 N solution of HCl in $Et_2O$ (2 mL) at 0 °C for 2 h.

*Compound **12a** × **HCl***: Crystallized from 2-propanol as light yellow prisms, mp 123.5–131.5 °C, dec. $^1$H NMR ($CD_3OD$) $\delta$: 2.15 (m, 2H), 2.28–2.46 (m, 6H), 2.82 (s, 3H), 3.08 (s, 2H), 3.10 (s, 3H), 4.01 (br s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 22.59, 30.60, 37.86, 39.85, 45.95, 63.37, 77.37, 169.28 ppm. MS (ESI) $m/z$ $[M+H]^+$ Calcd for $C_{11}H_{18}N_2O_2$: 210.3. Found: 211.1. $C_{11}H_{19}ClN_2O_2$ (246.73): Calcd C, 53.55; H, 7.76; Cl, 14.37; N, 11.35. Found: C, 53.79; H, 7.51; Cl, 14.20; N, 11.48.

**4.1.16.3. 2′-Methyl-3′-oxo-spiro{8-azabicyclo[3.2.1]octane-3,5′-isoxazolidine} hydrochloride (12b × HCl).** The salt was obtained in 81% yield by treating a solution of **23** (148 mg, 0.50 mmol) in dioxane (1 mL) with a 4 N solution of HCl in dioxane (1.1 mL) at 0 °C for 2 h.

*Compound **12b** × **HCl***: Crystallized from 2-propanol as colorless prisms, mp 230.5–233 °C. $^1$H NMR ($CD_3OD$) $\delta$: 2.11 (br s, 4H), 2.22 (dd, $J$ = 3.3 and 14.9, 2H), 2.35 (d, $J$ = 14.0, 2H) 3.04 (s, 2H), 3.10 (s, 3H), 4.16 (br s, 2H) ppm. $^{13}$C NMR ($CD_3OD$) $\delta$: 24.66, 30.57, 38.58,

45.80, 54.93, 78.07, 169.24 ppm. MS (ESI) *m/z* $[M+H]^+$ Calcd for $C_{10}H_{16}N_2O_2$: 196.3. Found: 197.1. $C_{10}H_{17}ClN_2O_2$ (232.71): Calcd C, 51.61; H, 7.36; Cl, 15.23; N, 12.04. Found: C, 51.44; H, 7.55; Cl, 15.07; N, 12.18.

### 4.2. Binding and uptake studies[37]

#### 4.2.1. Binding to DAT

Frozen rat striata were homogenized in 50 volumes of Tris HCl 50 mM, pH 7.4, containing 120 mM NaCl and 5 mM KCl, and centrifuged at 27,000*g* for 18 min at 4 °C. Pellets were washed twice and finally resuspended in 300 volumes of ice-cold buffer and 250 μL aliquots of membranes suspensions were incubated with 2 nM [$^3$H]WIN 35,428 (Perkin Elmer, specific activity 85.9 Ci/mmol), in absence or presence of tested compounds. Non-specific binding was determined using 3 μM nomifensine. After 90 min at 4 °C, incubation was stopped by rapid filtration through GF/B fiber filters presoaked in 0.1% albumin.

#### 4.2.2. Binding to SERT

Frozen rat cortices were homogenized in 50 volumes of Tris HCl 50 mM, pH 7.4, containing 120 mM NaCl and 5 mM KCl, and centrifuged at 27,000*g* for 18 min at 4 °C. Pellets were washed twice and finally resuspended in 400 volumes of ice-cold buffer and 500 μL aliquots of membranes suspensions were incubated with [$^3$H]citalopram or [$^3$H]paroxetine (Perkin Elmer, 2 and 0.5 nM respectively, or at different concentrations for saturation studies, specific activities 70 and 22.9 Ci/mmol respectively), in absence or presence of tested compounds. Non-specific binding was determined using 10 μM (*S*)-citalopram. After 60 min at 25 °C, incubation was stopped by rapid filtration through GF/B fiber filters presoaked in 0.5% polyethylenimine.

#### 4.2.3. SERT-mediated synaptosomal uptake

Fresh rat cortices were rapidly dissected after decapitation, and homogenized in 20 volumes of 0.32 M sucrose, pH 7.4 in phosphate buffer. After centrifugation at 1000*g* for 5 min at 4 °C, supernatants were centrifuged again at 12,000*g* for 20 min at 4 °C, to obtain the synaptosomal fractions $P_2$. The $P_2$ pellets were resuspended in buffer containing 140 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1 mM $MgSO_4$, 10 mM glucose, 1.2 mM $CaCl_2$, 1.2 mM $Na_2HPO_4$, 20 mM Hepes, 0.3 mM ascorbic acid, 0.25 mM pargyline, pH 7.4. Aliquots of 500 μL (corresponding to 3 mg tissue) were preincubated for 7 min at 35 °C in the absence or presence of tested compounds. Non-specific uptake was determined using 10 μM (*S*)-citalopram. Uptake was started by the addition of 100 nM [$^3$H]-5-HT (Perkin Elmer, specific activity 28 Ci/mmol) and the reaction was stopped 5 min later by rapid filtration through cellulose esters filters, followed by two washing steps.

Filters radioactivity was counted in 4 mL of scintillation liquid (Ultima Gold MV, Perkin Elmer) using a scintillation counter (Tricarb 2800TR, Perkin Elmer). Saturation curves were analyzed using the one site binding equation built into GraphPad Prism, giving, as estimated parameters, the maximum number of binding sites ($B_{max}$) and the dissociation constant ($K_d$), with their S.E.M. To evaluate the statistical significance of the differences found in the absence or presence of inhibitors, we compared parameters by using the extra sum-of-squares *F* test included in the GraphPad Prism software.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.004.

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