European Journal of Medicinal Chemistry 55 (2012) 255–261

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Chemoenzymatic synthesis and evaluation of 3-azabicyclo[3.2.0]heptane derivatives as dopaminergic ligands

Reet Reinart-Okugbeni [a,b,1], Kerti Ausmees [c,1], Kadri Kriis [c], Franz Werner [c], Ago Rinken [a,b,**], Tõnis Kanger [c,*]

[a] Institute of Chemistry, University of Tartu, Ravila 14, 50411 Tartu, Estonia
[b] Competence Centre on Reproductive Medicine and Biology, Tiigi 61b, 50410 Tartu, Estonia
[c] Department of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

ARTICLE INFO

Article history:
Received 7 October 2011
Received in revised form
9 July 2012
Accepted 17 July 2012
Available online 24 July 2012

Keywords:
Kinetic resolution
CAL-B
3-Azabicyclo[3.2.0]heptane derivatives
Dopamine receptor activity

ABSTRACT

New 3-azabicyclo[3.2.0]heptane derivatives were synthesized using a multicomponent reaction. Racemic compounds were efficiently resolved by kinetic resolution with immobilized lipase B of *Candida antarctica* (Novozym 435). The obtained compounds demonstrated greater binding affinity at D_{2L} and D_3 dopamine receptors compared to D_1 binding sites, and individual enantiomers of the same compound possessed distinct affinities.

1. Introduction

Dopamine is a monoamine neurotransmitter and its receptors are distributed throughout the body, both in CNS and in the periphery. According to their structural differences and signaling properties, dopamine receptors are divided roughly into D_1-like and D_2-like receptors, of which D_1-like receptors are comprised of D_1 and D_5 subtypes and D_2-like receptors include D_2, D_3 and D_4 subtypes. Disturbances in brain dopamine receptor signaling lead to such medical conditions as Parkinson's disease, schizophrenia and many other diseases resulting from genetic or environmental factors [1].

The structure–activity relationship of efficient dopamine receptor modulators indicates that a wide variety of structural units may be needed for the activity. However, a rigid heteroatom containing a bicyclic scaffold is often present in compounds with a therapeutic potential (Fig. 1) [2].

Substituents at the azabicyclo template are usually different and unique for every ring system. The ring size of the bicyclic heterocycle is a crucial feature. 3-Azabicyclo[3.1.0]hexane **1** derivatives with sulfonamide [3] or triazol [4] are selective dopamine D_3 antagonists. 3-Azabicyclo[3.2.1]octane **2** benzamide derivative SSR181507 [5] and indolyl-substituted bicyclic compounds [6] are D_2 antagonists. Benzamides of 3-azabicyclo[3.3.1]nonane **3** were found to be non-selective and showed nearly identical binding to dopamine D_2 and D_3 receptors [7]. Compounds with a 3-azabicyclo [3.2.0]heptane structure **4** have been shown to bind dopamine D_2-like receptors [8]. Our recent paper describes the synthesis of tetrasubstituted 3-azabicyclo[3.2.0]heptane derivatives **9** (Scheme 1) [9]. The method is characterized by a high diastereoselectivity, simplicity and a wide substrate scope. The starting unsaturated aldehyde **5** can be either aromatic, heteroaromatic or aliphatic, and secondary amine **7** can be either acyclic or cyclic. In addition, the primary hydroxyl group is a possible site for the further derivatization of the compound. This multicomponent cascade reaction is highly diastereoselective. The cascade involved an aza-Michael addition of benzyl aminocrotonate **6** to an iminium-activated α,β-unsaturated aldehyde, followed by the intramolecular Michael addition and an intramolecular Mannich-type ester enolate attack to iminium ion. The initially formed bicyclic ester **8** was unstable under reaction conditions and it was reduced in a one-pot procedure with $LiAlH_4$ to form the target **9**.

From the synthetic aspect, the access to 3-azabicyclo[3.2.0] heptanes is limited mainly to [2 + 2] photocycloaddition methods

* Corresponding author. Tel.: +372 6204371; fax: +372 6202828.
** Corresponding author. Institute of Chemistry, University of Tartu, Ravila 14, 50411 Tartu, Estonia. Tel.: +372 7375249; fax: +372 7375264.
E-mail addresses: ago.rinken@ut.ee (A. Rinken), kanger@chemnet.ee (T. Kanger).
[1] These authors have contributed equally.

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.07.025

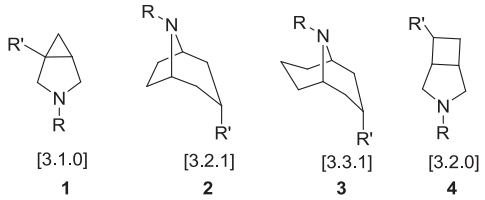


Fig. 1. Azabicyclo scaffolds found among dopamine receptor modulators.

[10,11] or Au- [12,13] or Pt-mediated [14] cycloisomerization. Their asymmetric synthesis has been even less explored. To the best of our knowledge, crystallization via ditoluyl tartaric acid salt [15] and Ir-catalyzed asymmetric allylic amination followed by Pt-catalyzed cyclization [16] are the only examples described in the literature so far.

Herein, we report the synthesis of various new derivatives of 3-azabicyclo[3.2.0]heptanes, a general method for the kinetic resolution of the obtained racemic products by lipase-catalyzed reaction, and assess the binding of obtained individual enantiomers at their potential biological target, dopamine receptors. 3-azabicyclo[3.2.0]heptanes are more likely to block D_2-like receptors [8], which is beneficial for antipsychotic drugs used i.e. for treating schizophrenia or related disorders [1]. Therefore the aim of the study was to determine selectivity profile of the synthesized 3-azabicyclo[3.2.0]heptane derivatives and find moieties that would substantially affect ligand binding to three dopamine receptor subtypes, D_1, D_{2L} and D_3.

2. Results and discussion

2.1. Lipase-catalyzed kinetic resolution of 3-azabicyclo[3.2.0] heptane derivatives

The stereodetermining step of the cascade in the synthesis of heterocycle **9** is the first aza-Michael addition. Our several attempts to use asymmetric organocatalytic approaches, such as iminium catalysis with chiral secondary amines (such as diaryl prolinol derivatives) or hydrogen bonding catalysts (quinidine, cinchonine, (*R*)-TRIP and chiral thiourea derivatives) afforded a racemic product. The same result was obtained with the auxiliary-based methods, where enantiomerically pure aminocrotonate derivatives were used in the multicomponent reaction.

As chemical methods of the enantioselective synthesis failed, we addressed this problem in the enzymatic reactions. It is well known that lipases are efficient enzymes for the acylation of alcohols [17]. The lipase-catalyzed kinetic resolution of the racemic secondary or even primary alcohols [18] is a widely used method for the synthesis of enantiomeric alcohols. We used immobilized lipase B of *Candida antarctica* (Novozym 435) as an acylating agent for the kinetic resolution of enantiomers of 3-azabicyclo[3.2.0] heptane **9** derivatives in EtOAc at room temperature (Scheme 2 and Table 1).

In addition to the previously known racemic compounds **9a**–**c** [9], the new derivatives **9d**, **9e**, **9f** and **9g** were synthesized and resolved enzymatically (the structures are depicted in Table 2). The method allowed us to obtain both enantiomers needed for the following pharmacological studies. In most cases, the enantioselectivity of the resolution was not high enough and a repeated resolution strategy was used. It afforded both enantiomers with sufficient enantiomeric excess. An acylated (B)-enantiomer (typically (−)-enantiomer) and nonacylated (A)-enantiomer (typically (+)-enantiomer) were separated by column chromatography and acylated (B)-isomer was hydrolyzed under alkaline conditions. Exceptions were the compounds **9e** and **9f**, with *p*-bromophenyl and pyridyl substituents, respectively. They behaved to the contrary, affording an (−)-enantiomer as nonacylated and (+)-enatiomer as acylated compounds.

For the determination of the absolute configuration the *N*-tosyl derivative **11** of 3-azabicyclo[3.2.0]heptane was synthesized and resolved (Scheme 3).

Kinetic resolution and hydrolysis of the obtained acylated B-enantiomer afforded a highly crystalline compound. Its absolute configuration was determined by using X-ray diffraction as (1*S*, 2*R*, 5*R*, 6*R*, 7*R*)-configuration (Fig. 2). It is assumed that all B-enantiomers of azabicycles that have been acylated by Novozym 435 possess the same configuration as shown.

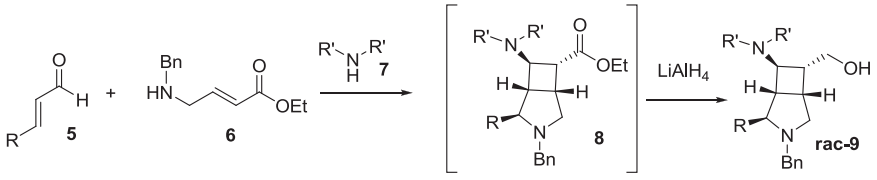
2.2. Ligand binding to dopamine receptors

Some azabicycloheptyl compounds, similar to the title compounds, have been shown to bind to D_2 and D_3 subtypes of dopamine receptors with a slight preference for the D_3 subtype [8]. Therefore, we saw dopamine receptors as a potential target for the present 3-azabicyclo[3.2.0]heptane derivatives and decided to test the compounds binding affinities for three different dopamine receptor subtypes, namely D_1, D_{2L} and D_3 receptors. In our earlier studies with dopaminergic compounds various host cell lines for different receptor subtypes, both human and rat, had been used, mainly for what was available [19,20]. However, the membrane environment [21,22] as well as the origin of the receptor gene [23] might have a substantial impact on ligand binding and signal transduction properties, so we created a single host (HEK293) based cell lines stably expressing individual subtypes of human dopamine receptors (D_1, D_{2L} and D_3), which is described in detail in the Experimental Section (Sections 4.3 and 4.4).

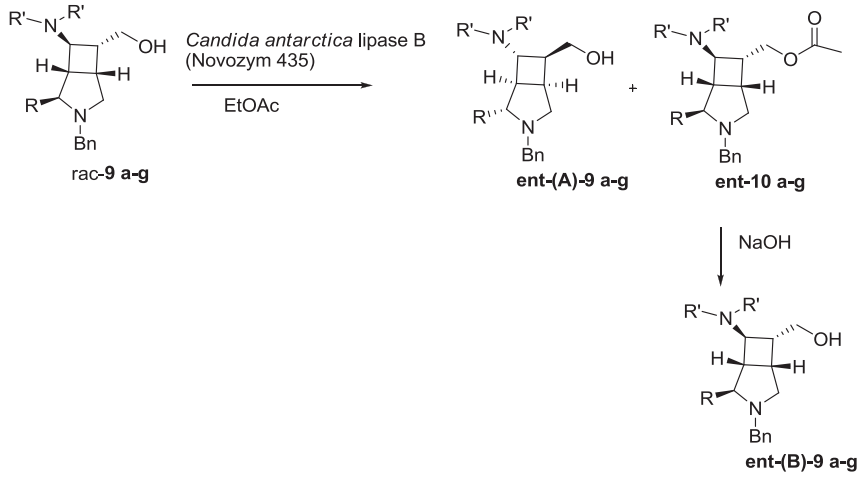
All tested 3-azabicyclo[3.2.0]heptane derivatives showed a preference for D_2-like receptors (see Table 2 for results and for the structures of tested compounds). None of them reached the affinity of apomorphine, but were comparable with dopamine. On the other hand, several B-enantiomers had better D_{2L} over D_1 selectivity compared to the reference compounds, but none achieved the level of D_3/D_{2L} selectivity of dopamine.

(B)-Enantiomers had substantially higher affinities (4- to 40-fold) toward D_2-like receptors compared to (A)-enantiomers, while the influence of the compounds absolute configuration on the affinities for the D_1 receptor was much less pronounced.

The addition of bromine to the phenyl moiety at position 2 of the core structure slightly improved the affinity for D_1 receptors (compare the compounds **9e** and **9a**). The highest affinity



Scheme 1. A multicomponent synthesis of racemic tetrasubstituted 3-azabicyclo[3.2.0]heptane derivatives.



Scheme 2. Kinetic resolution of 3-azabicyclo[3.2.0]heptane **9** derivatives.

compound of the current study was compound (B)-**9b**,which contained a pyrrolidinyl moiety at position 7 and a phenyl substituent at position 2, of which the pyrrolidinyl group seemed to improve the binding to D_{2L}/D_3 receptors, while the binding to D_1 receptors remains the same (compare (B)-**9b** and (B)-**9a**). The substitution of the pyrrolidine ring by a piperidine decreased both the affinity and selectivity (compounds (B)-**9b** and (B)-**9g**).

Comparing methyl and phenyl groups, the latter improved the affinity both for D_1 and D_{2L}/D_3 receptors, 10- and 50-fold, respectively (compare the compounds (B)-**9d** and (B)-**9b**). Generally, phenyl at position 2 increased the affinity for D_1 receptors irrespective of the substituents at position 7 (see compounds **9a**, **9b**, **9e** and **9f**). Significant selectivity was detected for only one compound, (B)-**9b**, having 80- to 90-fold higher affinity for D_2-like receptors compared to the D_1 receptor subtype.

For all the other higher affinity enantiomers, the preference for D_{2L} and D_3 receptors (between 10- and 30-fold) compared to D_1 was low. The exception was compound (B)-**9c** with a methyl at position 2 which bound very weakly to D_1 receptors while maintaining a considerable affinity for D_{2L} and D_3 receptors, increasing thereby the selectivity for D_2-like receptors to more than 40. The lack of selectivity between D_2 and D_3 receptor subtypes, as we encountered here, is a common feature for dopaminergic compounds and finding ligands that are specific for the D_3 rather than the D_2 receptor is one of the major challenges in dopaminergic receptor pharmacology [24]. In the current study however the goal was to identify the compounds preference for selected dopamine receptor subtypes. The binding studies confirmed our hypothesis that the newly synthesized 3-azabicyclo [3.2.0]heptane derivatives preferentially bind to D_2-like dopamine receptors.

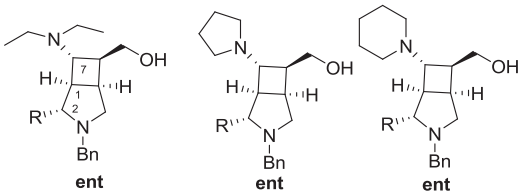
Table 1
Kinetic resolution of 3-azabicyclo[3.2.0]heptane **9** derivatives.

Entry	Compound	R	NR_2'	ee of (A)-enantiomer	ee of (B)-enantiomer
1	**9a**	Ph	NEt_2	99	97
2	**9b**	Ph	Pyrrolidinyl	96	99
3	**9c**	Me	NEt_2	98	94
4	**9d**	Me	Pyrrolidinyl	99	99
5	**9e**	*p*-BrPh	NEt_2	97	96
6	**9f**	2-pyridyl	NEt_2	97	99
7	**9g**	Ph	Piperidinyl	95	99

3. Conclusions

Enantiomers of 3-azabicyclo[3.2.0]heptane **9** derivatives were efficiently resolved by kinetic enzymatic resolution with immobilized lipase B of *Candida antarctica* (Novozym 435). The pharmacological evaluation of the obtained compounds revealed that all

Table 2
Binding affinities and binding selectivity of 3-azabicyclo[3.2.0]heptane derivatives **9a**–**g** ((A)- and (B)-enantiomers) to selected subtypes of the human dopamine receptors stably expressed in human embryonic kidney (HEK293) cells.



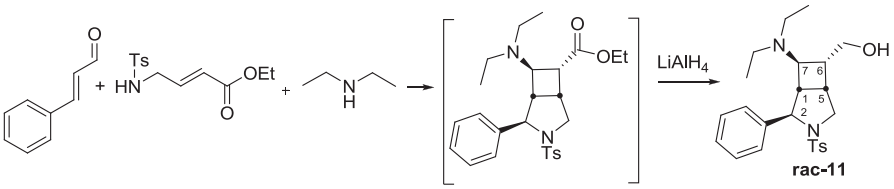
(A)-9a: R = Ph **(A)-9 b: R = Ph** **(A)-9 g: R = Ph**
c: R = Me **d: R = Me**
e: R = p-BrPh
f: R = 2-pyridyl

(B)-9 a-g are enantiomers of corresponding (A)-**9** compounds

Compounds	K_i [a] (μM)			Selectivity[b]		
	D_1	D_{2L}	D_3	D_{2L}/D_1	D_3/D_1	D_3/D_{2L}
(A)-**9a**	42 ± 7	12 ± 2	13.2 ± 0.4	3.4	3.2	0.9
(B)-**9a**	22 ± 8	1.7 ± 0.3	1.3 ± 0.2	13	17	1.3
(A)-**9b**	40.8 ± 0.7	7.4 ± 0.3	11 ± 2	5.5	3.6	0.6
(B)-**9b**	22 ± 3	0.25 ± 0.02	0.28 ± 0.04	90	80	0.9
(A)-**9c**	>100	38 ± 2	21 ± 9	>7	>10	1.8
(B)-**9c**	>100	3.9 ± 0.4	1.77 ± 0.01	>40	>85	2.2
(A)-**9d**	>100	66 ± 7	36 ± 8	>2	>4	1.8
(B)-**9d**	>100	16 ± 3	11 ± 1	>10	>15	1.5
(A)-**9e**	7 ± 1	5 ± 1	7.93 ± 0.02	1.4	0.9	0.7
(B)-**9e**	8 ± 1	2.0 ± 0.1	2.14 ± 0.01	3.9	3.6	0.9
(A)-**9f**	>100	42 ± 7	36 ± 10	>3	>3	1.2
(B)-**9f**	>100	12 ± 1	8.8 ± 0.3	>10	>15	1.4
(A)-**9g**	44 ± 2	7 ± 1	5.3 ± 0.3	6.4	8.3	1.3
(B)-**9g**	49 ± 0.1	2.0 ± 0.3	1.5 ± 0.1	25	34	1.4
Dopamine	12 ± 3	2.1 ± 0.2	0.015 ± 0.004	5.7	800	140
Apomorphine	0.66 ± 0.02	0.05 ± 0.01	0.010 ± 0.004	13	65	4.8

[a] K_i value in μM represents mean ± SEM from at least two independent experiments carried out in duplicates.

[b] Selectivities for D_{2L} over D_1 (D_{2L}/D_1), for D_3 over D_1 (D_3/D_1) and for D_3 over D_{2L} (D_3/D_{2L}) receptors are calculated as a ratio of $K_i(D_1)/K_i(D_{2L})$, $K_i(D_1)/K_i(D_3)$ and $K_i(D_{2L})/K_i(D_3)$, respectively. Selectivities marked as >are rough estimations due to very low affinity for the particular subtype.



Scheme 3. Synthesis of N-tosyl-3-azabicyclo[3.2.0]heptane derivative **11.**

compounds studied showed a moderate preference for D_2-like receptors and, not surprisingly, the binding affinity depended on the enantiomeric form of the tested 3-azabicyclo[3.2.0]heptane derivative.

4. Experimental section

Full assignment of ^1H and ^{13}C chemical shifts is based on the 1D and 2D FT NMR spectra on a Bruker Avance^{III} 400 instruments. Deuterosolvent peaks ($CHCl_3$ $\delta = 7.27$, $CDCl_3$ $\delta = 77.00$) or TMS peak was used as chemical shift references. Mass spectra were obtained on a Shimadzu GCMS-QP2010 spectrometer in GC/MS mode (EI, 70 eV). High resolution mass spectra were recorded on LTQ Orbitrap (Thermo Electron). IR spectra were recorded on Perkin–Elmer Spectrum BX FTIR spectrometer. X-ray diffraction data was collected on a Bruker SMART X2S at 200 K.

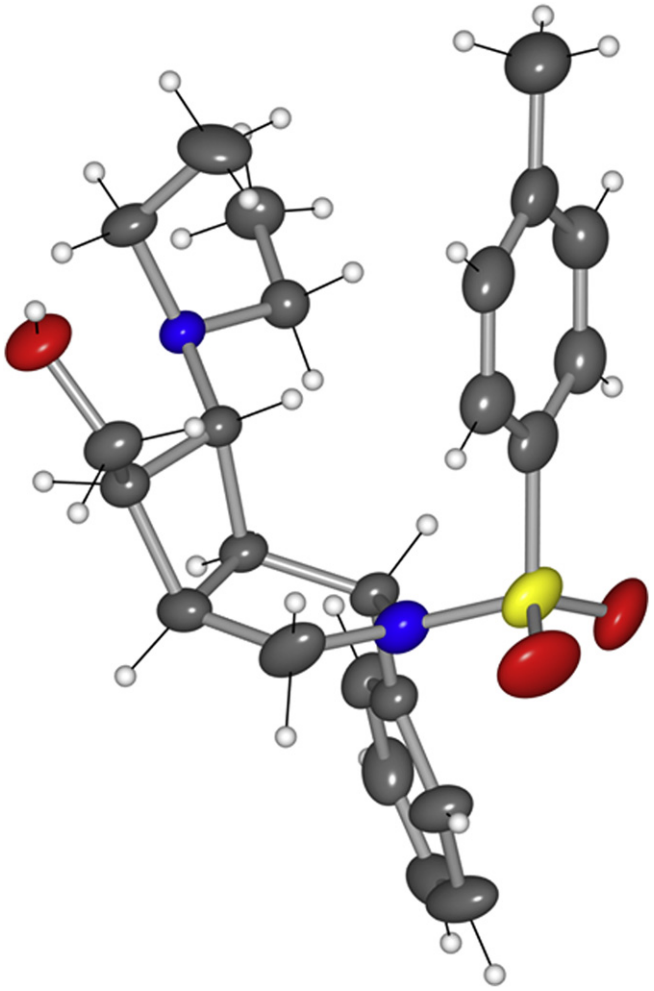


Fig. 2. Molecular moiety in the crystal structure of N-tosyl-3-azabicyclo[3.2.0]heptane derivative **11**. For clarity one formula unit is shown ($Z' = 2$). Displacement ellipsoids are drawn at the 50% probability level.

Reactions sensitive to oxygen or moisture were conducted under argon atmosphere in flame-dried glassware. Anhydrous dichloromethane was freshly distilled with CaH_2 and anhydrous tetrahydrofuran with $LiAlH_4$. Commercial reagents were generally used as received. Petroleum ether used had bp 40–60 °C.

4.1. General procedure for the synthesis of racemic *9*

To a solution of the corresponding aldehyde **5** (0.4 mmol) in anhydrous CH_2Cl_2 (1.0 mL) with molecular sieves (4Å) dialkyl amine **7** (0.4 mmol) and *N*-benzylaminocrotonate **6** (0.2 mmol) were added. The mixture was stirred at room temperature for 17–42 h. The mixture was concentrated in vacuum and the crude bicyclic ester **8** was reduced with $LiAlH_4$ (0.8 mmol) in anhydrous THF (1.0 mL). After 3 h the reaction mixture was cooled to 0 °C and the reaction was quenched by the addition of water and an aqueous solution of 4 M aq NaOH. The mixture was dried over K_2CO_3. The crude product was purified by chromatography on silica gel affording bicyclic alcohol **9**.

4.1.1. (3-Benzyl-2-exo-methyl-7-exo-pyrrolidin-1-yl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol ***9d***

Yield: 51%, off-white solid mp 102–104 °C. IR (KBr): 3191, 3062, 3028, 2958, 2909, 2800, 1748, 1632, 1494, 1454, 1331, 1240, 1174, 1152, 1128, 1075, 1029, 899, 786, 752, 699 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.31 (m, 4H), 7.30–7.24 (m, 1H), 3.85 (d, $J = 13.1$ Hz, 1H), 3.78 (dd, $J = 11.6$, 2.0 Hz, 1H), 3.63 (d, $J = 13.1$ Hz, 1H), 3.62 (dd, $J = 11.5$, 5.1 Hz, 1H), 3.07 (q, $J = 6.6$ Hz, 1H), 3.00 (dd, $J = 10.6$ Hz, 1H), 2.97 (ddd, $J = 9.8$, 7.9, 6.2 Hz, 1H), 2.87 (dd, $J = 5.7$, 3.9 Hz, 1H), 2.63 (dd, $J = 10.5$, 6.2 Hz, 1H), 2.45 (m, 5H), 2.31 (dd, $J = 8.1$, 3.7 Hz, 1H), 1.83–1.73 (m, 4H), 0.84 (d, $J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.23, 128.78, 128.48, 127.19, 63.77, 61.85, 60.12, 53.77, 51.20, 50.50, 47.57, 40.50, 34.31, 23.32, 11.70. HRMS (*m*/*z*): $[M + H^+]$ calcd for $(C_{19}H_{29}N_2O)^+$, 301.22744; found, 301.22748.

4.1.2. (3-Benzyl-7-exo-diethylamino-2-exo-p-bromophenyl-3-azabicyclo[3.2.0]hept-6-endo-yl) methanol ***9e***

Yield: 67%, pale yellow solid mp 43–45 °C. IR (KBr): 3131, 2969, 2906, 2859, 2801, 1945, 1905, 1809, 1639, 1586, 1487, 1468, 1450, 1368, 1343, 1325, 1294, 1195, 1130, 1010, 981, 814, 735, 701, 523 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.46–7.40 (m, 2H), 7.33 (m, 2H), 7.27 (m, 1H), 7.25–7.20 (m, 2H), 6.81 (m, 2H), 3.87 (s, 1H), 3.81 (dd, $J = 11.4$, 2.9 Hz, 1H), 3.70 (dd, $J = 11.4$, 6.1 Hz, 1H), 3.46 (d, $J = 13.4$ Hz, 1H), 3.28 (d, $J = 13.5$ Hz, 1H), 3.25 (dd, $J = 6.3$, 4.8 Hz, 1H), 3.08 (dd, $J = 16.3$, 8.3 Hz, 1H), 2.97 (dd, $J = 10.8$, 1.4 Hz, 1H), 2.74 (dd, $J = 10.9$, 6.8 Hz, 1H), 2.70 (dd, $J = 8.4$, 4.7 Hz, 1H), 2.55 (q, $J = 7.2$ Hz, 5H), 0.98 (t, $J = 7.2$ Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.34, 131.19, 130.26, 128.59, 128.43, 127.16, 121.39, 70.04, 62.43, 61.79, 54.74, 51.17, 47.63, 41.95, 40.09, 34.36, 10.59. HRMS (*m*/*z*): $[M + H^+]$ calcd for $(C_{24}H_{31}BrN_2O)^+$, 443.16925; found, 443.16862.

4.1.3. (3-Benzyl-7-exo-diethylamino-2-exo-pyridyl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol ***9f***

Yield: 53%, off-white solid mp 92–93 °C. IR (KBr): 3136, 2974, 2939, 2913, 2829, 1589, 1568, 1490, 1474, 1448, 1432, 1368, 1338,

1288, 1233, 1190, 1174, 1151, 1127, 1079, 1019, 992, 852, 779, 764, 747, 707, 650, 604 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.57 (ddd, J = 0.7, 1.7, 4.8, 1H), 7.59 (td, J = 1.8, 7.7, 1H), 7.34–7.14 (m, 6H), 6.90 (d, J = 7.8, 1H), 4.01 (s, 1H), 3.80 (dd, J = 3.0, 11.4, 1H), 3.66 (dd, J = 6.0, 11.5, 1H), 3.51 (d, J = 13.2, 1H), 3.41 (d, J = 13.2, 1H), 3.29 (dd, J = 4.7, 6.2, 1H), 3.14 (m, 1H), 3.13 (d, J = 8.6, 1H), 2.99 (d, J = 8.5, 1H), 2.76 (dd, J = 4.6, 7.7, 1H), 2.57 (q, J = 7.2, 4H), 2.53–2.49 (m, 1H), 0.99 (t, J = 7.2, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.57, 149.16, 138.63, 135.97, 128.78, 128.30, 127.11, 122.87, 122.11, 72.44, 62.45, 61.91, 55.10, 52.03, 47.04, 42.00, 39.88, 34.78, 10.55. HRMS (m/z): [M + H^+] calcd for $(C_{23}H_{31}N_3O)^+$, 366.25399; found, 366.25322.

4.1.4. (3-Benzyl-2-exo-phenyl-7-exo-piperidin-1-yl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol **9g**

Yield: 30%, off-white solid mp 148–151 °C. IR (KBr): 3183, 3065, 3029, 2924, 2782, 1601, 1490, 1452, 1368, 1339, 1261, 1236, 1122, 1027, 861, 777, 750, 711, 704 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.27 (m, 6H), 7.23 (m, 2H), 6.92 (m, 2H), 3.91 (s, 1H), 3.83 (dd, J = 11.4, 2.6 Hz, 1H), 3.69 (dd, J = 11.4, 5.9 Hz, 1H), 3.42 (d, J = 13.6 Hz, 1H), 3.31 (d, J = 13.6 Hz, 1H), 3.14 (m, 1H), 3.00 (dd, J = 10.7, 1.1 Hz, 1H), 2.82–2.75 (m, 3H), 2.56 (m, 1H), 2.41–2.20 (m, 4H), 1.61–1.50 (m, 4H), 1.45 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.07, 138.61, 128.71, 128.65, 128.35, 128.03, 127.44, 127.03, 70.50, 66.11, 62.64, 54.60, 51.17, 51.09, 46.91, 39.83, 34.74, 25.46, 24.39. HRMS (m/z): [M + H^+] calcd for $(C_{25}H_{33}N_2O)^+$, 377.25874; found, 377.25854.

4.2. General procedure of enzymatic kinetic resolution of **9**

Lipase B of *C. antarctica* (Novozym 435) (50 mg) was added to a solution of the racemic compound **9** (50 mg) in EtOAc (1.0 mL). The resulting mixture was stirred occasionally at room temperature and the reaction was monitored by TLC (about a conversion 50%). The typical time of the reaction varied from 4.5 to 7 h, then the lipase was filtered off and the filtrate concentrated under reduced pressure. This mixture was purified by chromatography on silica gel affording alcohol (A)-**9** and ester **12**. As the enantiomeric excess of ester **12** could not be determined by chiral HPLC, it was hydrolyzed to (B)-**9**, with 4 M NaOH in MeOH by stirring 3 h at room temperature. The enantiomeric excess of (A)-**9** and (B)-**9** was determined by HPLC (Chiralcel AS or Lux Amylose-2).

4.2.1. (3-Benzyl-7-exo-diethylamino-2-exo-phenyl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol **9a**

The enantiomeric excess was determined by HPLC, Chiralcel AS, Hex:iPrOH 98:2 with 0.1% Et_2NH, 1.0 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 11.6, *ee* 99%, [α] = +10 (c 0.82, CH_2Cl_2) and acylated (B)-enantiomer t_R = 16.3, *ee* 95%, [α] = −10 (c 0.47, CH_2Cl_2).

4.2.2. (3-Benzyl-2-exo-phenyl-7-exo-pyrrolidin-1-yl-3-azabicyclo [3.2.0]hept-6-endo-yl) methanol **9b**

The enantiomeric excess was determined by HPLC, Chiralcel AS, Hex:iPrOH 97:3 with 0.1% Et_2NH, 1.0 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 11.5, *ee* 96%, [α] = +10 (c 1.22, CH_2Cl_2) and acylated (B)-enantiomer t_R = 15.5, *ee* 99%, [α] = −11 (c 1.46, CH_2Cl_2).

4.2.3. (3-Benzyl-7-exo-diethylamino-2-exo-methyl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol **9c**

The enantiomeric excess was determined by HPLC, Lux Amylose-2, Hex:iPrOH 98:2 with 0.1% Et_2NH, 1.0 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 22.6, *ee* 99%, [α] = +31 (c 0.16, CH_2Cl_2) and acylated (B)-enantiomer t_R = 18.2, *ee* 94%, [α] = −28 (c 0.61, CH_2Cl_2).

4.2.4. (3-Benzyl-2-exo-methyl-7-exo-pyrrolidin-1-yl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol **9d**

The enantiomeric excess was determined by HPLC, Lux Amylose-2, Hex:iPrOH 97:3 with 0.1% Et_2NH, 1.5 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 16.6, *ee* 99%, [α] = +30 (c 0.31, CH_2Cl_2) and acylated (B)-enantiomer t_R = 13.9, *ee* 99%, [α] = −29 (c 0.48, CH_2Cl_2).

4.2.5. (3-Benzyl-7-exo-diethylamino-2-exo-p-bromophenyl-3-azabicyclo[3.2.0]hept-6-endo-yl)methanol **9e**

The enantiomeric excess was determined by HPLC, Chiralcel AS, Hex:iPrOH 98:2 with 0.1% Et_2NH, 1.0 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 12.2, *ee* 96% and acylated (B)-enantiomer t_R = 17.4, *ee* 99%, [α] = +4.9 (c 2.93, CH_2Cl_2).

4.2.6. (3-Benzyl-7-exo-diethylamino-2-exo-pyridyl-3-azabicyclo [3.2.0]hept-6-endo-yl)methanol **9f**

The enantiomeric excess was determined by HPLC, Chiralcel AS, Hex:iPrOH 98:2 with 0.1% Et_2NH, 1.0 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 23.1, *ee* 97%, [α] = −30 (c 1.97, CH_2Cl_2) and acylated (B)-enantiomer t_R = 29.4, *ee* 99%, [α] = +29.5 (c 1.53, CH_2Cl_2).

4.2.7. (3-Benzyl-2-exo-phenyl-7-exo-piperidin-1-yl-3-azabicyclo [3.2.0]hept-6-endo-yl) methanol **9g**

The enantiomeric excess was determined by HPLC, Chiralcel AS, Hex:iPrOH 97:3 with 0.1% Et_2NH, 1.0 mL/min, UV 254 nm, nonacylated (A)-enantiomer t_R = 10.2, *ee* 95%, [α] = +19 (c 0.37, CH_2Cl_2) and acylated (B)-enantiomer t_R = 14.5, *ee* 99%, [α] = −20 (c 0.37, CH_2Cl_2).

4.2.8. 7-(Exo-(diethylamino)-2-exo-phenyl-3-tosyl-3-azabicyclo [3.2.0]heptan-6-endo-yl)methanol **11**

To a solution of *trans*-cinnamaldehyde (0.4 mmol) in anhydrous CH_2Cl_2 (1.0 mL) diethyl amine (1.0 mmol) and *N*-tosylaminocrotonate (0.2 mmol) were added in the presence of molecular sieves (4Å). The mixture was stirred at room temperature for 20 h. The mixture was concentrated in vacuum and the crude bicyclic ester was reduced with $LiAlH_4$ (0.8 mmol) in anhydrous THF (1.0 mL). After 3 h the reaction mixture was cooled to 0 °C and was quenched by the addition of water and an aqueous solution of 4 M aq NaOH. The mixture was dried over K_2CO_3. The crude product was purified by chromatography on silica gel affording bicyclic alcohol **11** (smp 115–121 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, J = 7.1 Hz, 6H), 2.32 (s, 4H), 2.58–2.48 (m, 5H), 2.68–2.63 (m, 1H), 2.73–2.68 (m, 1H), 3.16–3.06 (m, 1H), 3.36 (dd, J = 11.3, 8.3 Hz, 1H), 3.75–3.62 (m, 2H), 3.87 (dd, J = 11.3, 1.9 Hz, 1H), 4.83 (s, 1H), 7.08–6.98 (m, 4H), 7.23–7.14 (m, 3H), 7.36–7.32 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 10.24, 21.41, 34.53, 40.03, 41.71, 47.17, 49.27, 61.58, 62.54, 68.97, 126.70, 127.02, 127.53, 128.50, 129.11, 136.13, 140.43, 142.77. HRMS (ESI^+): calculated for $(C_{24}H_{32}N_2O_3S)^+$ 248.2134 [M^+], found 248.2134.

4.3. Production of cell lines expressing human D_1, D_{2L} and D_3 dopamine receptors

We created a human embryonic kidney cells (HEK293) based stable lines expressing individual subtypes of human dopamine receptors (D_1, D_{2L} and D_3). Shortly, HEK293 cells (American Type Culture Collection, Rockville, MD) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (PAA Laboratories) supplemented with 10% fetal bovine serum (Gibco®), 100 U/mL penicillin and 100 μg/mL streptomycin (PAA Laboratories). Cells were grown at 37 °C in a humidified incubator with 5% CO_2. The pcDNA3.1+ expression vectors (Invitrogen) containing the desired gene of

human wild type dopamine receptor (*DRD1*, *DRD2L* and *DRD3*) were purchased from the Missouri S&T cDNA Resource Center. For transfection, cells were seeded on 6-well plates, cultured 24 h to reach ~90% confluence and transfected with 4 μg of DNA per well using Lipofectamine™ 2000 (Invitrogen) according to manufacturer's instructions. To obtain stable lines cells were maintained and passed for 2 weeks in the presence of 800 μg/mL geneticin (G418). Four to six G418 resistant colonies per each receptor subtype were selected and after five further passages the clonal cultures were tested for receptor expression by radioligand binding. Henceforth the cells were maintained in the presence of 400 μg/mL of G418. The clonal cell lines with similar receptor densities were further tested for their binding of known dopaminergic ligands and then used in experiments with the test compounds.

4.4. Radioligand binding and competition binding of 3-azabicyclo [3.2.0]heptane derivatives

The binding characteristics of the new cell lines were assessed by radioligand binding and competition binding of known dopaminergic ligands (dopamine and apomorphine). The radioligands [^{3}H]SCH23390 and [^{3}H]raclopride have high affinity for D_1-like and D_2-like dopamine receptors, respectively, which was also evident from our saturation binding experiments (data shown below). All ligand binding experiments were done on membrane suspensions, prepared as follows. The cells were centrifuged at 800 × *g* at room temperature and the pellet stored at −80 °C. The frozen pellets were melted on ice and washed by homogenization with a tissue homogenizer (Coleparmer Labgen 125) for 30 s in ice-cold PBS and centrifugation at 800 × *g* for 5 min at 4 °C. The pellet was re-homogenized in 50 mM Tris–HCl buffer, (pH = 7.4) and centrifuged at 30,000 × *g* for 20 min followed by a second resuspension and homogenization step. The latter homogenization and centrifugation steps were repeated once and the final pellet was homogenized in incubation buffer (IB: 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.4) typically at a concentration of 1×10^7 cells/mL (corresponding to ~0.5 mg protein/mL quantified using Bio-Rad protein assay Kit (Bio-Rad Laboratories) with BSA as a standard). The membrane preparations were stored at −80 °C until further testing.

Receptor expression and affinity of radioligands was determined as described earlier [25,26] with some modifications. Reactions were carried out in U-bottom 96-well plates in a final volume of 250 μL in IB and incubated at 25 °C for 60–90 min. In all assays 150 μL of membrane suspension was added to wells containing 50 μL of radioligand and 50 μL of competing unlabeled ligand (or buffer) at their five times final concentration. Receptor-positive clones were identified by one-point assay at a saturating concentration of [^{3}H]SCH23390 or [^{3}H]raclopride (Perkin Elmer) with or without an unlabeled dopaminergic antagonist, 1 μM (+)-butaclamol (Sigma–Aldrich). Receptor bound radioactivity ("specific binding") was calculated as a difference between radioactivity bound in the absence ("total binding") and in the presence ("non-specific binding") of an unlabeled antagonist. In saturation binding experiments the concentration of radioligand was varied from 0.2 to 10 nM and "non-specific binding" was determined in the presence of 1 μM (+)-butaclamol. Dissociation constants obtained, 0.5 nM for [^{3}H]SCH23390 binding to D_1 receptor and 0.7 nM for [^{3}H]raclopride binding to both D_{2L} and D_3 receptors, were in good agreement with the data reported earlier for these radioligands and receptors [27,28]. For 8-point competition assay the concentration of radioligand was fixed to near K_d value (~1 nM) and concentration of the test compounds was varied from 0.3 mM to 0.1 nM, the incubation buffer was supplemented with 1 mM DTT. Reactions were initiated by addition of membrane suspension (5×10^4 to 5×10^5 cells/assay for different receptor subtypes).

All the reactions were stopped by filtration through thick GF/B glass fiber filter mats (Whatham) using a FilterMate Harvester (Model D961962, Perkin Elmer). After five washes with ice-cold phosphate buffer (20 mM K-phosphate, 100 mM NaCl, pH 7.4), filter mats were dried in a microwave oven and impregnated with a MeltiLex™ B/HS scintillant (Wallac) using a MeltiLex® Heatsealer (Wallac). Filter-bound radioactivity was counted using a Wallac MicroBeta TriLux 1450 LSC Luminescence Counter (Perkin Elmer).

All pharmacological data were analyzed by means of non-linear least squares regression analysis using the commercial program GRAPHPAD PRISM™ 4.03 (GraphPad Software Inc.). Data were fit to one-site binding curve and inhibition constant values (K_i) calculated according to Cheng–Prusoff equation [29] (in Table 2 represented as means ± SEM from at least two independent experiments carried out in duplicates).

Acknowledgments

The authors thank the Estonian Science Foundation (Grants Nos. 8289 and 8314), the Ministry of Education and Research (Grant No. 0140060s12 and 0180032s12) and EU European Regional Development Fund (3.2.0101.08-0017, 3.2.0101.11-0029, 30020) for financial support.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.07.025.

References

[1] J.-M. Beaulieu, R.R. Gainetdinov, The physiology, signaling, and pharmacology of dopamine receptors, Pharm. Rev. 63 (2011) 182–217.
[2] C.M. Marson, New and unusual scaffolds in medicinal chemistry, Chem. Soc. Rev. 40 (2011) 5514–5533.
[3] F. Micheli, D. Hamprecht, G. Bonanomi, R. Di Fabio, D. Donati, G. Gentile, et al., [3-Azabicyclo[3.1.0]hex-1-yl]phenyl-benzenesulfonamides as selective dopamine D3 antagonists, Bioorg. Med. Chem. Lett. 20 (2010) 5491–5494.
[4] F. Micheli, L. Arista, G. Bonanomi, F.E. Blaney, S. Braggio, A.M. Capelli, et al., 1,2,4-Triazolyl azabicyclo[3.1.0]hexanes: a new series of potent and selective dopamine D(3) receptor antagonists, J. Med. Chem. 53 (2010) 374–391.
[5] R. Depoortere, D. Boulay, G. Perrault, O. Bergis, M. Decobert, D. Françon, et al., SSR181507, a dopamine D2 receptor antagonist and 5-HT1A receptor agonist. II: behavioral profile predictive of an atypical antipsychotic activity, Neuropsychopharmacology 28 (2003) 1889–1902.
[6] N.M. Paul, M. Taylor, R. Kumar, J.R. Deschamps, R.R. Luedtke, A.H. Newman, Structure–activity relationships for a novel series of dopamine D2-like receptor ligands based on N-substituted 3-aryl-8-azabicyclo[3.2.1]octan-3-ol, J. Med. Chem. 51 (2008) 6095–6109.
[7] R.H. Mach, P.S. Hammond, Y.S. Huang, B.O. Yang, Y.P. Xu, J.T. Cheney, et al., Structure–activity relationship studies of N-(9-benzyl)-9-azabicyclo[3.3.1] nonan-3-beta-yl benzamide analogues for dopamine D-2 and D-3 receptors, Med. Chem. Res. 9 (1999) 355–373.
[8] K. Drescher, A. Haupt, L. Unger, S.C. Turner, W. Braje, R. Grandel, Azabicycloheptyl Compounds Suitable for Treating Disorders that Respond to Modulation of the Dopamine D3 Receptor, U.S. Patent WO 2006040176, 2006.
[9] K. Kriis, K. Ausmees, T. Pehk, M. Lopp, T. Kanger, A novel diastereoselective multicomponent cascade reaction, Org. Lett. 12 (2010) 2230–2233.
[10] T. Bach, C. Pelkmann, K. Harms, Facial diastereoselectivity in the [2 + 2]-photocycloaddition of chiral vinylglycine-derived N, N-diallyl amines, Tetrahedron Lett. 40 (1999) 2103–2104.
[11] T. Bach, C. Kruger, K. Harms, The stereoselective synthesis of 2-substituted 3-azabicyclo[3.2.0]heptanes by intramolecular [2 + 2]-photocycloaddition reactions, Synthesis (2000) 305–320.
[12] M.R. Luzung, P. Mauleo, F.D. Toste, Gold(I)-catalyzed [2 + 2]-cycloaddition of allenenes, J. Am. Chem. Soc. 129 (2007) 12402–12403.
[13] Y.T. Lee, Y.K. Kang, Y.K. Chung, Au(I)-catalyzed cycloisomerization reaction of amide- or ester-tethered 1,6-enynes to bicyclo[3.2.0]hept-6-en-2-ones, J. Org. Chem. 74 (2009) 7922–7934.

[14] T. Matsuda, S. Kadowaki, M. Murakami, Synthesis of 3-acyl-4-alkenylpyrrolidines by platinum-catalyzed hydrative cyclization of allenynes, Helv. Chim. Acta 89 (2006) 1672–1680.
[15] N. Bischof, E. Buschmann, M. Henningsen, G.A. Krei, R. Munschauer, T. Pfeiffer, et al., Method for Producing (+)-Exo-6-phenyl-3-azabicyclo-[3.2.0]heptanes, U.S. Patent WO2000023423 A1, 2000.
[16] J.B. Xia, W.B. Liu, T.M. Wang, S.L. You, Enantioselective synthesis of 3-azabicyclo[4.1.0]heptenes and 3-azabicyclo[3.2.0]heptenes by Ir-catalyzed asymmetric allylic amination of N-tosyl propynylamine and Pt-catalyzed cycloisomerization, Chem. Eur. J. 16 (2010) 6442–6446.
[17] K. Faber, Biotransformations in Organic Chemistry, Springer, Heidelberg, 2004.
[18] C. Oger, Z. Marton, Y. Brinkmann, V. Bultel-Poncé, T. Durand, M. Graber, et al., Lipase-catalyzed regioselective monoacetylation of unsymmetrical 1,5-primary diols, J. Org. Chem. 75 (2010) 1892–1897.
[19] R. Reinart, Z. Gyulai, S. Berényi, S. Antus, A. Vonk, A. Rinken, et al., New 2-thioether-substituted apomorphines as potent and selective dopamine D2 receptor agonists, Eur. J. Med. Chem. 46 (2011) 2992–2999.
[20] A. Sipos, C. Csutorás, S. Berényi, A. Uustare, A. Rinken, Synthesis and neuropharmacological characterization of 2-O-substituted apomorphines, Bioorg. Med. Chem. 16 (2008) 4563–4568.
[21] A. Rinken, J. Harro, L. Engström, L. Oreland, Role of fluidity of membranes on the guanyl nucleotide-dependent binding of cholecystokinin-8S to rat brain cortical membranes, Biochem. Pharmacol. 55 (1998) 423–431.
[22] A. Rinken, Subtype-specific changes in ligand binding properties after solubilization of muscarinic receptors from baculovirus-infected Sf9 insect cell membranes, J. Pharm. Exp. Ther. 272 (1995) 8–14.
[23] J.E. Leysen, W. Gommeren, J. Mertens, W.H. Luyten, P.J. Pauwels, M. Ewert, et al., Comparison of in vitro binding properties of a series of dopamine antagonists and agonists for cloned human dopamine D2S and D2L receptors and for D2 receptors in rat striatal and mesolimbic tissues, using [125I] 2′-iodospiperone, Psychopharmacology 110 (1993) 27–36.
[24] J. Carlsson, R.G. Coleman, V. Setola, J.J. Irwin, H. Fan, A. Schlessinger, et al., Ligand discovery from a dopamine D(3) receptor homology model and crystal structure, Nat. Chem. Biol. (2011) 1–10.
[25] A. Uustare, R. Reinart, A. Rinken, Modulation of dopamine D1 receptor signaling by adenosine A1 receptors in Sf9 cells requires expression of Gi proteins, Neurosci. Lett. 406 (2006) 169–173.
[26] J. Harro, A. Terasmaa, M. Eller, A. Rinken, Effect of denervation of the locus coeruleus projections by DSP-4 treatment on [3H]-raclopride binding to dopamine D2 receptors and D2 receptor–G protein interaction in the rat striatum, Brain Res. 976 (2003) 209–216.
[27] A. Rinken, S. Ferré, A. Terasmaa, C. Owman, K. Fuxe, Serotonergic agonists behave as partial agonists at the dopamine D2 receptor, NeuroReport 10 (1999) 493–495.
[28] Y. Cao, W.C. Sun, L. Jin, K.Q. Xie, X.Z. Zhu, Activation of adenosine A1 receptor modulates dopamine D1 receptor activity in stably cotransfected human embryonic kidney 293 cells, Eur. J. Pharmacol. 548 (2006) 29–35.
[29] Y.-C. Cheng, W.H. Prusoff, C. Yung-Chi, Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, Biochem. Pharmacol. 22 (1973) 3099–3108.