Bioorganic & Medicinal Chemistry 17 (2009) 1445–1455

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Relationships between the structure of 6-substituted 6,8-diazabicyclo [3.2.2]nonan-2-ones and their σ receptor affinity and cytotoxic activity

Ralph Holl [a], Dirk Schepmann [a], Patrick J. Bednarski [b], Renate Grünert [b], Bernhard Wünsch [a,*]

[a] Institut für Pharmazeutische und Medizinische Chemie der Westfälischen Wilhelms-Universität Münster, Hittorfstraße 58-62, D-48149 Münster, Germany
[b] Institut für Pharmazie der Ernst-Moritz-Arndt-Universität Greifswald, Friedrich-Ludwig-Jahn-Straße 17, D-17489 Greifswald, Germany

ARTICLE INFO

Article history:
Received 17 November 2008
Revised 5 January 2009
Accepted 10 January 2009
Available online 15 January 2009

Keywords:
Diazabicyclo[3.2.2]nonanes
Sigma receptor ligands
Cytotoxicity
Structure–activity-relationships

ABSTRACT

A series of 2-oxo-6,8-diazabicyclo[3.2.2]nonane derivatives was prepared and the affinity towards σ_1 and σ_2 receptors was investigated by means of radioligand binding assays as well as their inhibition of the growth of six human tumor cell lines was studied. Starting from the enantiopure bicyclic ketones **3** and ent-**3** bridged piperazines with different residues in position 6 were synthesized. The N-6 allyl protective group was removed by a $RhCl_3$ catalyzed double bond isomerization and subsequent hydrolysis of the resulting enamide **8**. After acetalization the secondary amide **10** was alkylated and arylated. Structure affinity relationships show that a relatively large substituent, which has not necessarily to be an aromatic one, is required in position 6 for high σ_1 receptor affinity (e.g., **12** and ent-**12** with a dimethylallyl residue: K_i = 20 nM and 17 nM). Furthermore, it was shown that substituents that reduce the basicity of N-6 led to a severe decrease in σ_1 affinity. Growth inhibition experiments with six human tumor cell lines revealed that the allyl and benzyl substituted 6,8-diazabicyclo[3.2.2]nonan-2-one derivatives **5**, ent-**5** and ent-**14** are able to selectively inhibit the growth of the bladder cancer cell line 5637.

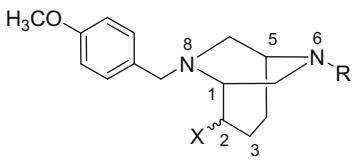
1. Introduction

The σ receptor was first described as an opioid receptor subtype.[1] However today it is accepted to be a distinct pharmacological entity, with the class of σ receptors being subdivided into σ_1 and σ_2 receptors.[2] Although their biochemical role as well as their mechanism of signal transduction are not yet completely understood so far, they have been implicated in a multitude of biological and phathophysiological processes such as cancer biology.[3]

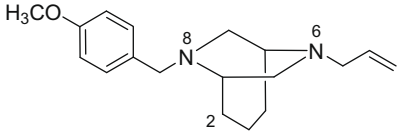
The σ_1 receptor has been cloned and shows no homology to any other mammalian protein but shows about 30% homology to the yeast enzyme sterol Δ^8/Δ^7-isomerase.[4] The cloned receptor is postulated to possess two transmembrane domains with the amino and carboxy termini on the intracellular side of the membrane. Mutation of the human σ_1 receptor revealed aspartate 126 and glutamate 172 to be crucial amino acids for ligand binding. These observations indicate that the binding domain of the receptor is in its intracellular carboxy terminus.[5,6] However, so far little is known about the exact structure of the ligand binding site of the membrane bound σ_1 receptor and the receptor features being important for ligand binding.

Glennon et al. reported on the structure affinity relationships of a series of phenylalkylamine derivatives with respect to their binding at σ_1 receptors and elaborated the features of these compounds being important for high σ_1 receptor binding.[7] According to the proposed two dimensional model, two hydrophobic substituents in different distances from a basic nitrogen atom, which is supposed to bind to a proton donor site (Asp 126 and/or Glu 172) of the receptor, are required for a high σ_1 receptor affinity (compare Fig. 2).

We recently reported on the synthesis and σ receptor affinity of a series of 6,8-diazabicyclo[3.2.2]nonane derivatives **1** (Fig. 1).[8] These compounds possess two basic nitrogen atoms, which in principal could both bind to the postulated proton donor site of the σ_1



1



2

Figure 1. General structure of the 6,8-diazabicyclo[3.2.2]nonane derivatives (**1**) and structure of the 2-unsubstituted compound **2**: Ligands for the σ_1 receptor.

* Corresponding author. Fax: +49 251 8332144.
E-mail address: wuensch@uni-muenster.de (B. Wünsch).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.01.012

receptor. However, the N-6 allyl derivatives **1** bearing a substituent in position 2 showed a considerably reduced affinity towards the σ_1 receptor compared to the unsubstituted compound **2** (K_i = 11 nM). These findings led to the conclusion that unfavorable interactions of the C-2 substituent arise with the σ_1 receptor protein when N-8 binds to the proton donor site (Fig. 2a). In case of N-6 binding to the proton donor site, the allyl group seems to produce a low free energy during interaction with the secondary hydrophobic region of the receptor (Fig. 2b). In order to further expand on these results a series of 6,8-diazabicyclo[3.2.2]nonan-2-one derivatives was synthesized bearing various N-6-substituents, which modify the hydrophobic interactions with the receptor protein and the basicity of the N-atom.

2. Chemistry

The synthesis of the central bicyclic building block **3** emanating from (*S*)-glutamate has been described in the literature.[8,9] Starting from the enantiomerically pure bicyclic ketone **3** the dimethyl acetal **4** was formed in methanol in the presence of trimethyl orthoformate and *p*-toluenesulfonic acid.[8,9] Reaction of **4** with $LiAlH_4$ led to the reduction of both lactam carbonyl moieties and a subsequent acetal cleavage yielded the bridged piperazine derivative **5** (Scheme 1). Hydrogenation of the allyl group of **4** gave the N-6 propyl derivative **6**, which was also reduced with $LiAlH_4$ and subsequently hydrolyzed with diluted HCl to yield the bicyclic ketone **7**.[8]

In order to introduce further substituents in position 6 the allyl group of **4** must be removed. The allyl group was cleaved by a $RhCl_3$ catalyzed isomerization and subsequent hydrolysis of the resulting enamide **8**, which yielded the secondary amide **9** (Scheme 2). After regeneration of the acetal moiety the secondary amide **10** was alkylated. Deprotonation with NaHMDS and subsequent reaction with dimethylallyl bromide and benzyl bromide yielded the dimethylallyl and benzyl derivatives **11** and **13**, respectively. Subsequent $LiAlH_4$ reduction and acetal cleavage afforded the basic piperazine derivatives **12** and **14**.

The arylation of the secondary amide **10** was achieved with iodobenzene in DMF in the presence of CuI and K_2CO_3 at 150 °C.[10] The N-8-phenyl derivative **15** was subsequently reduced and hydrolyzed to give the aniline derivative **16** (Scheme 3). Direct $LiAlH_4$ reduction and acetal cleavage of **10** yielded the secondary amine **17**, which was acylated with benzoyl chloride to form the benzamide **18**.

The enantiomers of the bicyclic ketones **5**, **7**, **12**, **14**, **16** and **18** were prepared in the same manner, starting with (*R*)-glutamate. Therefore, all possible stereoisomers of the described compounds were available for pharmacological evaluation. The purity of all compounds tested was evaluated by RP-HPLC analysis with UV detection at wavelength of 210 nm (Method 1) and 235 nm (Method 2). All compounds were found to have at least a purity of 95%.

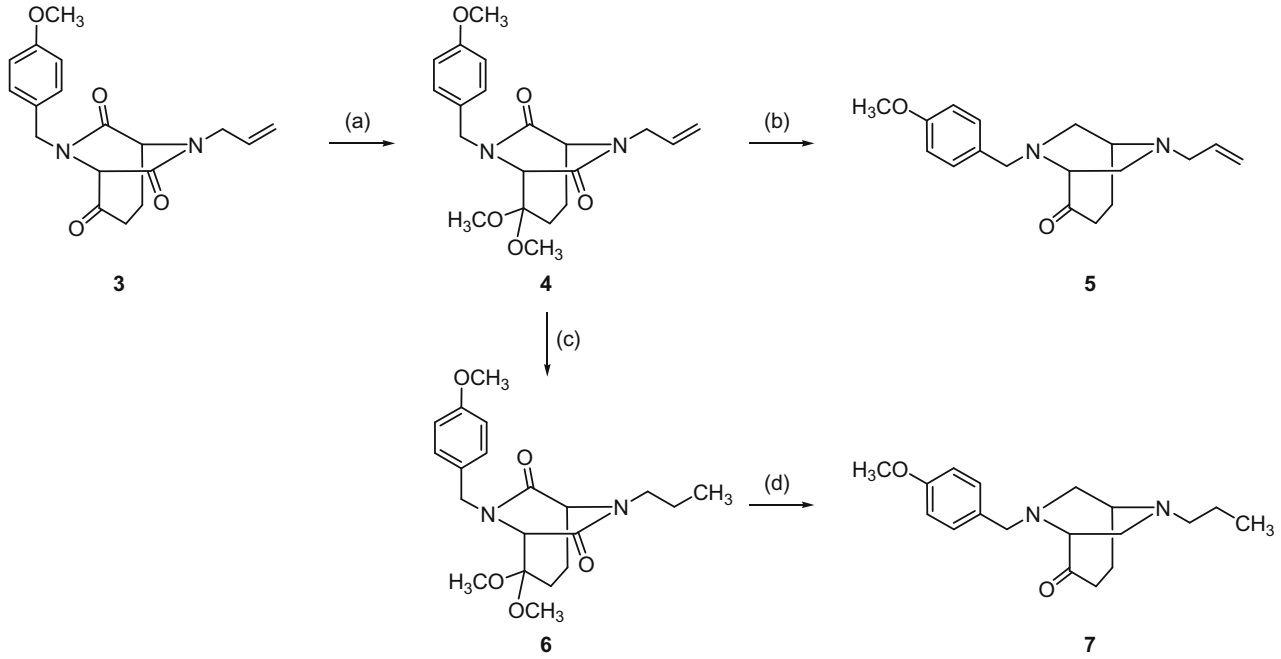
3. Pharmacological evaluation

3.1. Receptor binding studies

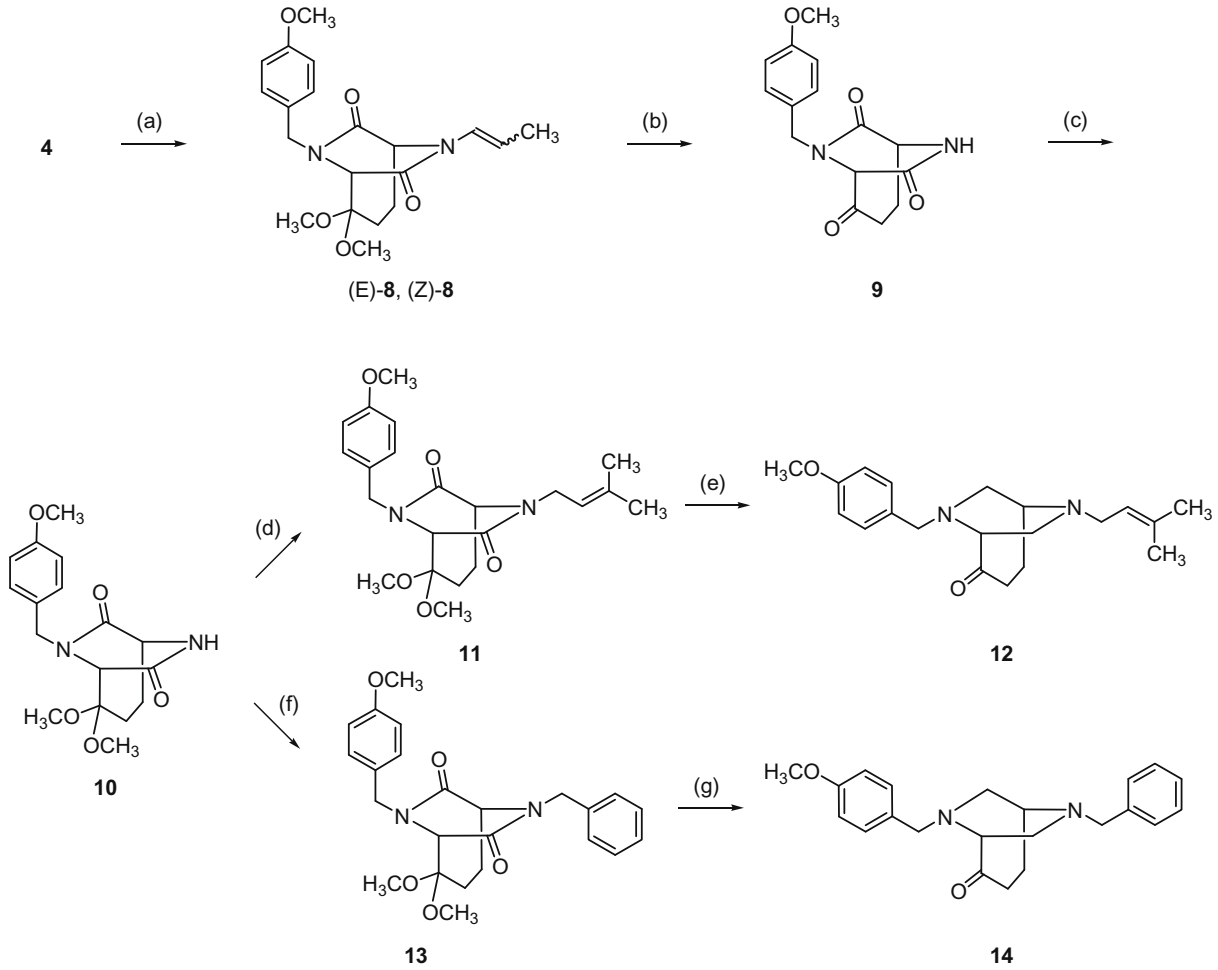
The σ receptor affinities of the synthesized compounds were determined in competition experiments with radioligands. Homogenates of guinea pig brains were used as receptor material in the σ_1 assay and the σ_1 selective ligand [^{3}H]-(+)-pentazocine was employed as radioligand. The non-specific binding was determined in the presence of a large excess of cold (+)-pentazocine. In the σ_2 assay homogenates of rat liver served as source for σ_2 receptors. The non-selective radioligand [^{3}H]-1,3-di(o-tolyl)guanidine was employed in the presence of an excess of non-tritiated (+)-pentazocine for selective occupation of σ_1 receptors. The non-specific binding of the radioligand was determined by performing the σ_2 assay in the presence of an excess of non-tritiated 1,3-di(o-tolyl)guanidine.[11,12]

3.2. Inhibition of cell growth of human tumor cell lines

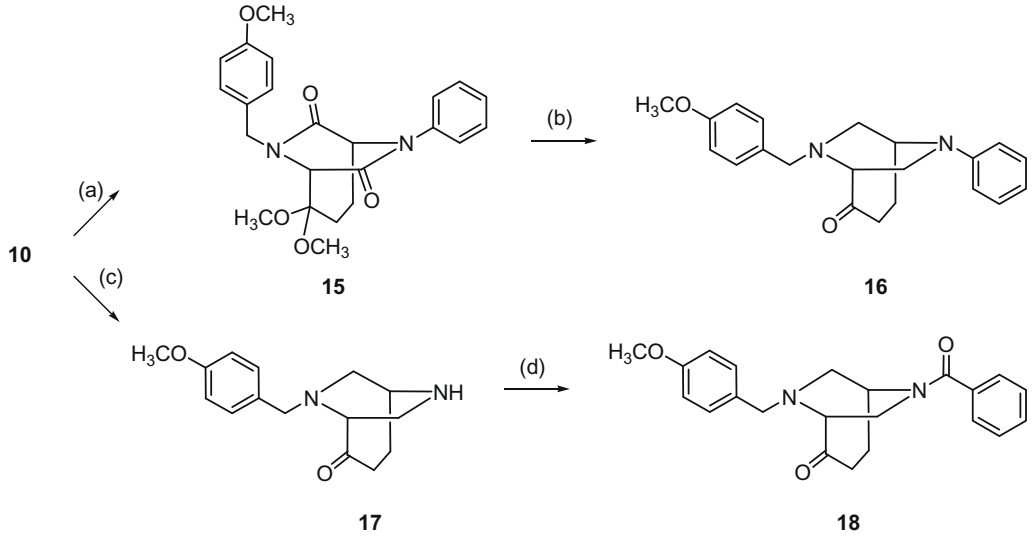
In the literature the overexpression of σ_1 and σ_2 receptors in human tumor cell lines has been reported.[6] Furthermore, some σ_2 agonists and σ_1 antagonists showed antiproliferative and cytotoxic effects in some tumor cell lines.[13] Therefore, the antiproliferative effects of the synthesized compounds were investigated in a panel of six human tumor cell lines, including the cell lines 5637



Scheme 1. Reagents and conditions: (a) $HC(OCH_3)_3$, MeOH, *p*-toluenesulfonic acid, reflux, 16 h, 99% (according to Ref. 8); (b) $LiAlH_4$, THF, reflux, 16 h, then 1 M HCl, 3 h, 50% (according to Ref. 8); (c) H_2, Pd/C, MeOH, rt, 1 h, 92%; (d) $LiAlH_4$, THF, reflux, 16 h, then 1 M HCl, 3 h, 34%.



Scheme 2. Reagents and conditions: (a) $RhCl_3$, DABCO, MeOH, reflux, 16 h, 66% (*E*)-**8**, 9% (*Z*)-**8;** (b) H_2O/HOAc, reflux 16 h, 24%; (c) $HC(OCH_3)_3$, MeOH, *p*-toluenesulfonic acid, reflux, 16 h, 80%; (d) NaHMDS, 1-bromo-3-methylbut-2-ene, Bu_4NI, THF, −78 °C, 82%; (e) $LiAlH_4$, THF, reflux, 16 h, then 1 M HCl, 3 h, 75%; (f) NaHMDS, benzyl bromide, Bu_4NI, THF, −78 °C, 67%; (g) $LiAlH_4$, THF, reflux, 16 h, then 1 M HCl, 3 h, 50%.



Scheme 3. Reagents and conditions: (a) Iodobenzene, CuI, K_2CO_3, DMF, 150 °C, 72 h, 72%; (b) $LiAlH_4$, THF, reflux, 16 h, then 1 M HCl, 3 h, 20%; (c) $LiAlH_4$, THF, reflux, 16 h, then 1 M HCl, 3 h, 59%; (d) benzoyl chloride, NEt_3, CH_2Cl_2, rt, 16 h, 61%.

(bladder cancer), RT-4 (bladder cancer), A-427 (small cell lung cancer), LCLC-103H (large cell lung cancer), MCF-7 (breast cancer) and DAN-G (pancreas cancer).

In the primary screening the tumor cells were exposed to 20 μM solutions of the test compounds at 37 °C. After a 96 h exposure the medium was removed and the density of adherent cells (living

cells) was measured by staining with crystal violet.[14] In Table 2 the cell growth inhibiting activity of the test compounds is expressed as the percent of living cells after 96 h in relation to a control without test compound.

4. Biological results and discussion

Table 1 shows the σ_1 and σ_2 receptor affinity of the synthesized compounds. Whereas the introduction of a carbonyl group in position 2 leads to a severe decrease in σ_1 affinity (**5**: K_i = 1600 nM) compared to the unsubstituted compound (**2**: K_i = 11 nM), the introduction of different substituents in position 6 reverse this effect. The N-6 propyl derivative **7** interacts better with the σ_1 receptor (K_i = 325 nM) than the allyl derivative **5** (K_i = 1600 nM). Increasing of the π-system of the allyl derivative **5** to a benzyl group (**14**) leads to an increased σ_1 receptor affinity (**14**: K_i = 53 nM). In this series of 6,8-diazabicyclo[3.2.2]nonan-2-ones the dimethylallyl derivative **12** shows the highest σ_1 affinity (K_i = 20 nM). Apparently, a relatively large lipophilic substituent, which is able to interact with the σ_1 receptor, is required in position 6 for high σ_1 receptor binding.

The effect of the stereochemistry of the bicyclic system on σ_1 receptor binding is inconsistent. Whilst in case of the allyl and propyl derivatives ent-**5** and ent-**7** show higher σ_1 affinity than their enantiomers derived from (*S*)-glutamate, the N-6 benzyl derivative **14** is the eutomer with respect to σ_1 receptor binding. The enantiomeric dimethylallyl substituted compounds **12** and ent-**12** display comparable affinities towards the σ_1 receptor.

The phenyl and benzoyl derivatives **16** and **18**, each with reduced basicity of N-6 because of the substituents, show considerably lower σ_1 receptor affinities than the other compounds of this series. This indicates that in this series of 6,8-diazabicyclo[3.2.2]nonan-2-ones the basicity of N-6 is crucial for σ_1 receptor binding and therefore this nitrogen atom is likely to bind to the proton donor site of the σ_1 receptor. After reduction of the N-6-basicity the other basic N-atom in position 8 presumably occupies the proton donor site of the σ_1 receptor protein. However, in this orientation (Fig. 2, part 2a) the unfavorable interaction of the carbonyl oxygen with the σ_1 receptor protein lead to low affinity of **16** and **18**.

These results further underline the proposed binding mode of the 2-oxo-6,8-diazabicyclo[3.2.2]nonan-2-one derivatives (Fig. 2b). When a basic N-6 is binding to the proton donor site, a relatively large lipophilic substituent in position 6 (e.g., **12**: dimethylallyl, **14**: benzyl) is required for favorable interactions with the secondary hydrophobic region (A) of the σ_1 receptor resulting in high σ_1 receptor affinity. The lipophilic N-6 substituent must not necessarily be an aromatic residue. This hypothesis of interaction of the bridged piperazine derivatives with the σ_1 receptor protein is in accordance with the observations made with σ_1 receptor ligands modified in position 2 of the bicyclic framework.[8]

Generally, the σ_2 receptor affinity of the bicyclic compounds is rather low. Therefore in particular the compounds with high σ_1 affinity show a very good selectivity for the σ_1 receptor over the σ_2 receptor.

Since some σ receptor ligands show considerable affinity towards the NMDA receptor, the affinity of the bridged piperazines towards the phencyclidine binding site of the NMDA receptor (pig brain cortex membrane preparations, [^{3}H]-MK801) was also determined.[12] However, at a concentration of 1 μM the replacement of the radioligand by the synthesized compounds was always lower than 25%. These results indicate high selectivity of the bicyclic σ_1 ligands over the PCP binding site of the NMDA receptor.

Table 2 shows the cell growth inhibiting properties of the synthesized compounds. At a concentration of 20 μM the N-6 allyl derivatives **5** and ent-**5** as well as the benzyl derivative ent-**14** selectively inhibit the growth of the bladder cancer cell line 5637 with modest potency, whilst the N-6 phenyl derivative ent-**16** inhibits unselectively the growth of most of the investigated cell lines including 5637, A-427, LCLC-103H and MCF-7. The growth of the other cell lines was reduced to a lower extent by these compounds. The selective inhibition of the growth of only few cell lines by **5**, ent-**5** and ent-**14** indicates a specific mechanism of growth inhibition.

The most potent σ_1 ligands of this series, the dimethylallyl compounds **12** and ent-**12** as well as the benzyl derivative **14**, possess K_i values of 20 nM, 17 nM and 53 nM, respectively. The cell growth inhibition of all six tumor cell lines together induced by **12**, ent-**12**, and **14** was rather low. However, looking selectively at the breast cancer cell line MCF-7 these three most potent σ_1 ligands represent the most potent growth inhibitors of this tumor cell line as well. Nevertheless, the potency of around 55% growth inhibition at a test compound concentration of 20 μM is still moderate. This might be due to the different abilities of the compounds to penetrate through the tumor cell membrane to attain the intracellularly located binding site of the σ_1 receptor. Furthermore, it should be considered that the σ_1 affinities of **12**, ent-**12**, and **14** are considerably lower than that of the cytotoxic σ_1 ligand haloperidol. A further explanation would be a potential agonistic or partial agonistic instead of antagonistic activity at σ_1 receptors.

5. Conclusion

6,8-Diazabicyclo[3.2.2]nonan-2-one derivatives show considerable affinity towards the σ_1 receptor. This affinity is strongly dependent on the substituent in position 6. Whereas an allyl moiety leads to a rather low affinity (**5**: K_i = 1600 nM), propyl and benzyl substituents increase the σ_1 affinity considerably. The dimethylallyl derivatives show the highest σ_1 affinity (**12**: K_i = 20 nM, ent-**12**: K_i = 17 nM) in this series of compounds.

Furthermore, substituents like a phenyl and a benzoyl group, which decrease the basicity of N-6, lead to almost complete loss of σ_1 receptor affinity in this series of 6,8-diazabicyclo[3.2.2]nonan-2-one derivatives. Obviously, basic properties are required in position 6 to attain high σ_1 receptor affinity.

Some of the synthesized bridged piperazine derivatives possess modest antiproliferative activity against some human tumor cell lines. However, further structure–activity investigations are required to establish the relationships between this antiproliferative activity and the σ_1 and/or σ_2 receptor affinity of these interesting compounds.

6. Experimental

6.1. Chemistry, general

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F254 plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, eluent, fraction size, R_f value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. Optical rotation: Polarimeter 341 (Perkin–Elmer); 1.0 dm tube; concentration *c* [g/100 mL], the unit of [α] [grad mL dm^{-1} g^{-1}]. MS: MAT GCQ (Thermo-Finnigan); IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Unity Mercury Plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. HPLC: Method 1: Merck Hitachi Equipment; UV detector: L-7400; autosampler:L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 μm); LiCroCART® 250-4 mm cartridge;

Table 1
σ_1 and σ_2 receptor affinity of the new 6,8-diazabicyclo[3.2.2]nonane derivatives and some reference compounds

Compound	N-6 substituent	σ_1 K_i ± SEM (nM)	σ_2 K_i ± SEM (nM)
5	Allyl	1600	9100
ent-**5**	Allyl	548	1900
7	Propyl	325	23%[a]
ent-**7**	Propyl	67 ± 8.2	1800
12	Dimethylallyl	20 ± 5.5	841
ent-**12**	Dimethylallyl	17 ± 4.6	1300
14	Benzyl	53 ± 2.1	704
ent-**14**	Benzyl	133 ± 23	3600
16	Phenyl	6250	3780
ent-**16**	Phenyl	5%[a]	5%[a]
18	Benzoyl	20%[a]	29%[a]
ent-**18**	Benzoyl	59%[a]	11%[a]
2	Allyl	11 ± 4.7[8]	203[8]
ent-**2**	Allyl	270[8]	335[8]
(+)-Pentazocine	—	4.2 ± 1.1	—
Di-*o*-tolylguanidine	—	61 ± 18	42 ± 17
haloperidol	—	3.9 ± 1.5	78 ± 2.3

[a] Inhibition of radioligand binding at a concentration of the test compound 1 μm.

flow rate: 1.000 mL/min; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: 0.0 min: 90.0% of A, 10.0% of B; 4.0 min: 90.0% of A, 10.0% of B; 29.0 min: 0.0% of A, 100.0% of B; 31.0 min: 0.0% of A, 100.0% of B; 31.5 min: 90.0% of A, 10.0% of B; 40.0 min: 90.0% of A, 10.0% of B. Method 2: Equipment: pump: HPLC pump 64 (Knauer); UV-Detector: Variable Wavelength Monitor (Knauer); data acquisition: D-2500 Chromato-Integrator (Merck Hitachi); column: LiChroCART® 250-4 with Superspher® 100 RP-18; flow rate: 0.6 mL/min; injection volume: 20.0 μL; detection: wavelength: 235 nm; stop time: 2 × t_R; method (2a) solvent: methanol/water = 75:25 + 0.1% triethylamine; method (2b) solvent: methanol/water = 65:35 + 0.1% triethylamine.

Table 2
Cell growth inhibitory activity (% of untreated control) of the 6,8-diazabicyclo[3.2.2]nonane derivatives in six human cancer cell lines[a]

	5637[b]	RT-4[c]	A-427[d]	LCLC-103H[e]	MCF-7[f]	DAN-G[g]
5	38	80	60	81	72	75
ent-**5**	19	76	50	64	65	69
7	119	101	94	102	69	117
ent-**7**	80	97	105	96	60	101
12	119	102	83	99	63	109
ent-**12**	72	100	81	86	57	99
14	62	89	64	91	56	89
ent-**14**	35	84	60	79	50	84
16	94	102	111	88	66	109
ent-**16**	17	102	37	47	43	62
18	86	102	68	64	72	79
ent-**18**	120	108	110	102	84	116
2	87	65	42	91	65	92
ent-**2**	90	71	55	82	74	87

[a] Relative cell growth [%] in relation to untreated control of the tumor cell lines after 96 h exposure to compound at 20 μM.
[b] Bladder cancer.
[c] Bladder cancer.
[d] Small cell lung cancer.
[e] Large cell lung cancer.
[f] Breast cancer.
[g] Pancreas cancer.

6.2. (+)-(1*S*,5*S*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-propyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (6)

The dimethyl acetal **4**[8] (140 mg, 0.37 mmol) was dissolved in methanol (30 mL) and 10% Pd/C (14 mg) was added. The mixture was stirred under hydrogen (balloon) for 1 h at rt. Then the suspension was filtered through Celite and the filtrate was concentrated in vacuo. The residue was purified by fc (Ø = 2 cm, *h* = 15 cm, cyclohexane/ethyl acetate = 2/1, 10 mL, R_f = 0.08) to give **6** as a colorless solid,

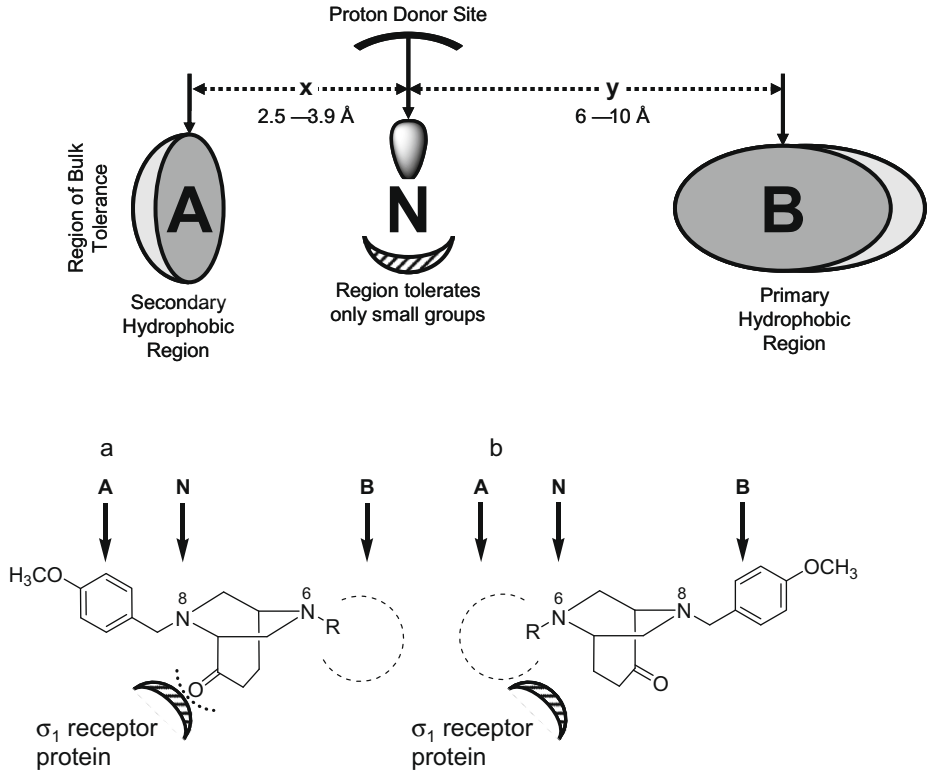


Figure 2. Pharmacophore model of σ_1 receptor ligands (modified according to Ref. 7) and hypothetical binding modes of the bridged piperazine derivatives at the σ_1 receptor protein. Binding mode (a) N-8 interacts with the proton donor site. Binding mode (b) N-6 interacts with the proton donor site.

mp 98 °C, yield 130 mg (92%). $C_{20}H_{28}N_2O_5$ (376.5). Purity by HPLC: method 1: t_R = 18.4 min, purity 99.1%. $[\alpha]_D^{20}$ = +35.7 (c = 0.15; CH_2Cl_2). MS (EI): m/z [%] = 376 (M, 1), 121 (CH_2PhOCH_3, 40), 101 ($H_2CCHC(OCH_3)_2$, 100). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.25 (t, J = 7.0 Hz, 3H, $NCH_2CH_2CH_3$), 1.46–1.62 (m, 2H, $NCH_2CH_2CH_3$), 1.74–1.86 (m, 2H, 3-H), 2.01–2.15 (m, 2H, 4-H), 3.15–3.23 (m, 1H, $NCH_2CH_2CH_3$), 3.17 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 3.41–3.50 (m, 1H, $NCH_2CH_2CH_3$), 3.77 (s, 3H, $ArOCH_3$), 3.88 (dd, J = 5.5/1.6 Hz, 1H, 5-H), 3.96 (d, J = 14.9 Hz, 1H, NCH_2Ar), 3.97 (s, 1H, 1-H), 5.18 (d, J = 14.9 Hz, 1H, NCH_2Ar), 6.83 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.11 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2940*** (m, $\nu_{C-H\ aliph.}$), 1675 (s, $\nu_{C=O\ amide}$), 1613 (m)/1512 (m, $\nu_{C=C\ arom.}$), 1457 (m, $\delta_{C-H\ aliph.}$), 1238 (m)/1038 (m, ν_{C-O}), 832 (w, $\Gamma_{p\text{-subst. arom.}}$).

6.3. (−)-(1*R*,5*R*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-propyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (ent-6)

As described for the preparation of **6**, the enantiomer ent-**4**[8] (100 mg, 0.27 mmol) was reacted with hydrogen (balloon) and 10% Pd/C (10 mg) in methanol (30 mL) to give ent-6 as a colorless solid, mp 98 °C, yield 76 mg (76%). $C_{20}H_{28}N_2O_5$ (376.5). Purity by HPLC: method 1: t_R = 18.5 min, purity 98.0%. $[\alpha]_D^{20}$ = −36.3 (c = 0.30; CH_2Cl_2).

6.4. (−)-(1*R*,5*S*)-8-(4-Methoxybenzyl)-6-propyl-6,8-diazabicyclo[3.2.2]nonan-2-one (7)

Under N_2 a 1.0 M solution of $LiAlH_4$ in THF (1.17 mL, 1.17 mmol) was added to an ice-cooled solution of **6** (110 mg, 0.29 mmol) in THF (40 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then 1.0 M HCl (10 mL) was added under ice-cooling and the mixture was stirred at 0 °C for 10 min and then refluxed for 3 h. After cooling down the mixture was alkalized with a saturated aqueous solution of $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 100/1, V = 10 mL, R_f = 0.11) to give **7** as a yellow oil, yield 30 mg (34%). $C_{18}H_{26}N_2O_2$ (302.4). Purity by HPLC: method 2a: t_R = 16.2 min, purity 99.3%; method 1: t_R = 16.0 min, purity 97.6%. $[\alpha]_D^{20}$ = −97.7 (c = 0.43; CH_2Cl_2). MS (EI): m/z [%] = 302 (M, 2), 181 (M–CH_2PhOCH_3, 3), 121 (CH_2PhOCH_3, 100). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.84 (t, J = 7.0 Hz, 3H, $NCH_2CH_2CH_3$), 1.36–1.46 (m, 2H, $NCH_2CH_2CH_3$), 1.68–1.78 (m, 1H, 4-H), 1.84–1.92 (m, 1H, 4-H), 2.27 (ddd, J = 13.3/8.6/1.6 Hz, 1H, 3-H), 2.46 (t, J = 7.0 Hz, 2H, $NCH_2CH_2CH_3$), 2.77 (dd, J = 10.2/2.3 Hz, 1H, piperazine-H), 2.81–2.88 (m, 2H, piperazine-H), 2.91 (dd, J = 11.0/2.3 Hz, 1H, piperazine-H), 2.97–3.01 (m, 1H, piperazine-H), 3.17 (t, J = 3.1 Hz, 1H, piperazine-H), 3.26 (ddd, J = 13.3/10.2/8.6 Hz, 1H, 3-H), 3.60 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.64 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.78 (s, 3H, $ArOCH_3$), 6.83 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.18 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2955 (m, $\nu_{C-H\ aliph.}$), 1708 (s, $\nu_{C=O\ ketone}$), 1611 (m)/1510 (s, $\nu_{C=C\ arom.}$), 1244 (m)/1033 (m, ν_{C-O}), 820 (w, $\Gamma_{p\text{-subst. arom.}}$).

6.5. (+)-(1S,5R)-8-(4-Methoxybenzyl)-6-propyl-6,8-diazabicyclo[3.2.2]nonan-2-one (ent-7)

As described for the preparation of **7**, the enantiomer ent-**6** (70 mg, 0.19 mmol) was reacted with $LiAlH_4$ (0.74 mL of a 1.0 M solution in THF, 0.74 mmol) in THF (30 mL) and afterwards hydrolyzed with 1.0 M HCl (5 mL) to give ent-**7** as a yellow oil, yield 39 mg (69%). $C_{18}H_{26}N_2O_2$ (302.4). Purity by HPLC: method 2a: t_R = 15.8 min, purity 99.1%; method 1: t_R = 15.9 min, purity 98.9%. $[\alpha]_D^{20}$ = +94.1 (c = 1.40; CH_2Cl_2).

6.6. (−)-(1*S*,5*S*,*E*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-(prop-1-en-1-yl)-6,8-diazabicyclo[3.2.2]nonan-7,9-dione ((*E*)-8) and (−)-(1*S*,5*S*,*Z*)-2,2-dimethoxy-8-(4-methoxybenzyl)-6-(prop-1-en-1-yl)-6,8-diazabicyclo[3.2.2]nonan-7,9-dione ((*Z*)-8)

Under N_2 the dimethyl acetal **4**[8] (229 mg, 0.61 mmol) was dissolved in methanol (30 mL). Then 1,4-diazabicyclo[2.2.2]octane (69 mg, 0.61 mmol) and rhodium(III) chloride trihydrate (16 mg, 0.06 mmol) were added and the mixture was heated to reflux for 16 h. Then a saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 2 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, V = 10 mL) to give (*E*)-**8** and (*Z*)-**8**.

(*E*)-**8** (R_f = 0.22): Colorless oil, yield 150 mg (66%). $C_{20}H_{26}N_2O_5$ (374.4). Purity by HPLC: method 1: t_R = 20.0 min, purity 97.9%. $[\alpha]_D^{20}$ = −23.3 (c = 0.39; CH_2Cl_2). MS (EI): m/z [%] = 374 (M, 23), 342 (M–$HOCH_3$, 28), 121 (CH_2PhOCH_3, 100), 101 ($H_2CCHC(OCH_3)_2$, 99). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.73 (dd, J = 7.0/1.6 Hz, 3H, $NCH{=}CHCH_3$), 1.74–1.90 (m, 2H, 3-H), 2.02–2.08 (m, 1H, 4-H), 2.11–2.18 (m, 1H, 4-H), 3.18 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.78 (s, 3H, $ArOCH_3$), 3.96 (d, J = 14.9 Hz, 1H, NCH_2Ar), 4.00 (s, 1H, 1-H), 4.39–4.42 (m, 1H, 5-H), 5.12 (dq, J = 14.1/7.0 Hz, 1H, $NCH{=}CHCH_3$), 5.22 (d, J = 14.9 Hz, 1H, NCH_2Ar), 6.83–6.88 (m, 1H, $NCH{=}CHCH_3$), 6.84 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.12 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2939 (m, $\nu_{C-H\ aliph.}$), 1680 (s, $\nu_{C=O\ amide}$), 1612 (m)/1512 (m, $\nu_{C=C\ arom.}$), 1422 (m, $\delta_{C-H\ aliph.}$), 1242 (m)/1102 (m)/1036 (m, ν_{C-O}), 809 (w, $\Gamma_{p\text{-subst. arom.}}$).

(Z)-**8** (R_f = 0.12): Colorless oil, yield 20 mg (9%). $C_{20}H_{26}N_2O_5$ (374.4). $[\alpha]_D^{20}$ = −25.0 (c = 0.25; CH_2Cl_2). MS (EI): m/z [%] = 374 (M, 4), 342 (M–$HOCH_3$, 8), 121 (CH_2PhOCH_3, 64), 101 ($H_2CCHC(OCH_3)_2$, 100). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.65 (dd, J = 7.0/2.3 Hz, 3H, $NCH{=}CHCH_3$), 1.81–1.93 (m, 2H, 3-H), 2.09–2.21 (m, 2H, 4-H), 3.19 (s, 3H, OCH_3), 3.29 (s, 3H, OCH_3), 3.79 (s, 3H, $ArOCH_3$), 4.00 (d, J = 14.9 Hz, 1H, NCH_2Ar), 4.03 (s, 1H, 1-H), 4.08 (dd, J = 6.3/1.6 Hz, 1H, 5-H), 5.23 (d, J = 14.9 Hz, 1H, NCH_2Ar), 5.43 (dq, J = 8.6/7.0 Hz, 1H, $NCH{=}CHCH_3$), 5.96–6.00 (m, 1H, $NCH{=}CHCH_3$), 6.85 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.15 (d, J = 8.6 Hz, 2H, 2″-$H_{4\text{-methoxybenzyl}}$, 6″-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2944 (m, $\nu_{C-H\ aliph.}$), 1681 (s, $\nu_{C=O\ amide}$), 1612 (m)/1512 (m, $\nu_{C=C\ arom.}$), 1419 (m, $\delta_{C-H\ aliph.}$), 1244 (m)/1101 (m)/1035 (m, ν_{C-O}), 808 (w, $\Gamma_{p\text{-subst. arom.}}$).

6.7. (+)-(1*R*,5*R*,*E*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-prop-1-en-1-yl-6,8-diazabicyclo[3.2.2]nonan-7,9-dione (ent-(*E*)-8) and (+)-(1*R*,5*R*,*Z*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-prop-1-en-1-yl-6,8-diazabicyclo[3.2.2]nonan-7,9-dione (ent-(*Z*)-8)

As described for the preparation of (*E*)-**8** and (*Z*)-**8**, the enantiomer ent-**4**[8] (130 mg, 0.35 mmol) was reacted with 1,4-diazabicyclo[2.2.2]octane (39 mg, 0.35 mmol) and rhodium(III) chloride trihydrate (9 mg, 0.03 mmol) in methanol (20 mL) to give ent-(E)-**8** and ent-(Z)-**8**.

ent-(*E*)-**8**: Colorless oil, yield 79 mg (61%). $C_{20}H_{26}N_2O_5$ (374.4). Purity by HPLC: method 1: t_R = 19.9 min, purity 97.9%. $[\alpha]_D^{20}$ = +22.7 (c = 0.63; CH_2Cl_2).

ent-(Z)-**8**: Colorless oil, yield 20 mg (15%). $C_{20}H_{26}N_2O_5$ (374.4). $[\alpha]_D^{20}$ = +24.1 (c = 0.22; CH_2Cl_2).

6.8. (+)-(1S,5S)-8-(4-Methoxybenzyl)-6,8-diazabicyclo[3.2.2]-nonane-2,7,9-trione (9)

(*E*)-**8** (170 mg, 0.45 mmol) was dissolved in a mixture of water/glacial acetic acid (1/1, 20 mL) and the mixture was heated

to reflux for 16 h. Then the mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø = 2 cm, h = 15 cm, ethyl acetate, 10 mL, R_f = 0.25) to give **9** as a colorless solid, mp 264 °C (decomposition), yield 31 mg (24%). $C_{15}H_{16}N_2O_4$ (288.3). Purity by HPLC: method 1: t_R = 13.4 min, purity 96.5%. $[\alpha]_D^{20}$ = +48.1 (c = 0.08; H_3CCN). MS (EI): m/z [%] = 288 (M, 4), 121 (CH_2PhOCH_3, 100). ^1H NMR ($CDCl_3$): δ [ppm] = 2.10–2.22 (m, 1H, 4-H), 2.40–2.46 (m, 1H, 3-H), 2.50–2.60 (m, 2H, 3-H (1H), 4-H (1H)), 3.79 (s, 3H, ArOCH_3), 4.10–4.14 (m, 1H, 5-H), 4.19 (d, J = 2.3 Hz, 1H, 1-H), 4.38 (d, J = 14.1 Hz, 1H, NCH_2Ar), 4.79 (d, J = 14.1 Hz, 1H, NCH_2Ar), 6.37 (s br, 1H, NH), 6.85 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.21 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3241 (m br, ν_{N-H}), 2973 (m, $\nu_{C-H\ aliph.}$), 1724 (m, $\nu_{C=O\ ketone}$), 1663 (s, $\nu_{C=O\ amide}$), 1613 (m)/1513 (m, $\nu_{C=C\ arom.}$), 1450 (m, $\delta_{C-H\ aliph.}$), 1248 (m)/1025 (m, ν_{C-O}), 833 (m, $\Gamma_{p\text{-subst. arom.}}$).

6.9. (−)-(1*R*,5*R*)-8-(4-Methoxybenzyl)-6,8-diazabicyclo[3.2.2] no nane-2,7,9-trione (ent-9)

As described for the preparation of **9**, the enantiomer ent-(E)-**8** (236 mg, 0.63 mmol) was reacted in a mixture of water/glacial acetic acid (1/1, 30 mL) to give ent-**9** as a colorless solid, mp 260 °C (decomposition), yield 77 mg (42%). $C_{15}H_{16}N_2O_4$ (288.3). Purity by HPLC: method 1: t_R = 13.4 min, purity 100%. $[\alpha]_D^{20}$ = −48.6 (c = 0.08; H_3CCN).

6.10. (+)-(1S,5S)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (10)

5Under N_2 trimethyl orthoformate (0.50 mL, 486 mg, 4.58 mmol) was added to a solution of **9** (120 mg, 0.42 mmol) and p-toluenesulfonic acid (158 mg, 0.92 mmol) in methanol (30 mL). The mixture was heated to reflux for 16 h. Then a saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 2 cm, h = 15 cm, cyclohexane/ethyl acetate = 1/2, V = 10 mL, R_f = 0.06) to give **10** as a colorless oil, yield 112 mg (80%). $C_{17}H_{22}N_2O_5$ (334.4). Purity by HPLC: method 1: t_R = 15.9 min, purity 95.9%. $[\alpha]_D^{20}$ = +60.7 (c = 0.22; CH_2Cl_2). MS (EI): m/z [%] = 334 (M, 6), 213 (M−CH_2PhOCH_3, 1), 121 (CH_2PhOCH_3, 40), 101 ($H_2CCHC(OCH_3)_2$, 100). ^1H NMR ($CDCl_3$): δ [ppm] = 1.77–1.90 (m, 2H, 3-H), 2.05–2.20 (m, 2H, 4-H), 3.18 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 3.78 (s, 3H, ArOCH_3), 3.91 (d, J = 2.3 Hz, 1H, 1-H), 3.93–3.97 (m, 1H, 5-H), 3.95 (d, J = 14.9 Hz, 1H, NCH_2Ar), 5.22 (d, J = 14.9 Hz, 1H, NCH_2Ar), 6.67 (d br, J = 5.5 Hz, 1H, NH), 6.85 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.14 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3239 (m br, ν_{N-H}), 2943 (m, $\nu_{C-H\ aliph.}$), 1679 (s, $\nu_{C=O\ amide}$), 1612 (m)/1512 (m, $\nu_{C=C\ arom.}$), 1436 (m, $\delta_{C-H\ aliph.}$), 1244 (m)/1035 (m, ν_{C-O}), 807 (w, $\Gamma_{p\text{-subst. arom.}}$).

6.11. (−)-(1*R*,5*R*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (ent-10)

As described for the preparation of **10**, the enantiomer ent-**9** (77 mg, 0.27 mmol) was reacted with trimethyl orthoformate (0.32 mL, 312 mg, 2.94 mmol) and p-toluenesulfonic acid (101 mg, 0.59 mmol) in methanol (30 mL) to give ent-**10** as colorless oil, yield 70 mg (78%). $C_{17}H_{22}N_2O_5$ (334.4). $[\alpha]_D^{20}$ = −61.8 (c = 0.13; CH_2Cl_2).

6.12. (−)-(1S,5S)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-(3-methylbut-2-en-1-yl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (11)

Under N_2 a solution of **10** (61 mg, 0.18 mmol) and tetrabutylammonium iodide (13 mg, 0.04 mmol) in THF (30 mL) was cooled to −78 °C. Then a 2.0 M solution of sodium hexamethyldisilazane in THF (0.10 mL, 0.20 mmol) was added dropwise. After stirring the mixture at −78 °C for 40 min, the 1-bromo-3-methylbut-2-ene (0.11 mL, 136 mg, 0.91 mmol) was added. The mixture was stirred at −78 °C for 1 h and was then allowed to warm to rt. After stirring the mixture at rt for 2 h, water was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 5 mL, R_f = 0.14) to give **11** as a colorless oil, yield 60 mg (82%). $C_{22}H_{30}N_2O_5$ (402.5). Purity by HPLC: method 1: t_R = 20.3 min, purity 99.4%. $[\alpha]_D^{20}$ = −10.9 (c = 0.59; CH_2Cl_2). MS (EI): m/z [%] = 402 (M, 5), 121 (CH_2PhOCH_3, 54), 101 ($H_2CCHC(OCH_3)_2$, 100). ^1H NMR ($CDCl_3$): δ [ppm] = 1.67 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.73 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.75–1.83 (m, 2H, 3-H), 1.97–2.07 (m, 2H, 4-H), 3.16 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 3.77 (s, 3H, ArOCH_3), 3.87 (dd, J = 5.5/2.3 Hz, 1H, 5-H), 3.91 (dd, J = 14.9/7.0 Hz, 1H, NC$H2$CH=C(CH_3)$_2$), 3.95 (d, J = 14.9 Hz, 1H, NCH_2Ar), 3.96 (s, 1H, 1-H), 4.05 (dd, J = 14.9/7.0 Hz, 1H, NC$H2$CH=C(CH_3)$_2$), 5.08–5.13 (m, 1H, $NCH_2CH{=}C(CH_3)_2$), 5.17 (d, J = 14.9 Hz, 1H, NCH_2Ar), 6.83 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.11 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2933 (m, $\nu_{C-H\ aliph.}$), 1678 (s, $\nu_{C=O\ amide}$), 1612 (m)/1513 (m, $\nu_{C=C\ arom.}$), 1450 (m, $\delta_{C-H\ aliph.}$), 1244 (m)/1035 (m, ν_{C-O}), 808 (w, $\Gamma_{p\text{-subst. arom.}}$).

6.13. (+)-(1*R*,5*R*)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-(3-methylbut-2-en-1-yl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (ent-11)

As described for the preparation of **11**, the enantiomer ent-**10** (66 mg, 0.20 mmol), tetrabutylammonium iodide (15 mg, 0.04 mmol), 2 M solution of sodium hexamethyldisilazane in THF (0.11 mL, 0.22 mmol) and 1-bromo-3-methylbut-2-ene (0.12 mL, 147 mg, 0.99 mmol) were reacted in THF (30 mL) to give ent-**11** as a colorless oil, yield 63 mg (79%). $C_{22}H_{30}N_2O_5$ (402.5). Purity by HPLC: method 1: t_R = 20.1 min, purity 96.8%. $[\alpha]_D^{20}$ = +10.9 (c = 0.26; CH_2Cl_2).

6.14. (−)-(1*R*,5*S*)-8-(4-Methoxybenzyl)-6-(3-methylbut-2-en-1-yl)-6,8-diazabicyclo[3.2.2]nonan-2-one (12)

Under N_2 a 1.0 M solution of $LiAlH_4$ (0.74 mL, 0.74 mmol) was added to an ice-cooled solution of **11** (74 mg, 0.18 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then 1.0 M HCl (5 mL) was added under ice-cooling and the mixture was stirred at 0 °C for 10 min and then refluxed for 3 h. After cooling down the mixture was alkalized with a saturated aqueous solution of $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, CH_2Cl_2/methanol = 100/1, V = 5 mL, R_f = 0.09) to give **12** as a yellow oil, yield 45 mg (75%). $C_{20}H_{28}N_2O_2$ (328.5). Purity by HPLC: method 2a: t_R = 19.5 min, purity 100%; method 1: t_R = 18.0 min, purity 99.8%. $[\alpha]_D^{20}$ = −77.8 (c = 0.34; CH_2Cl_2). MS (EI): m/z [%] = 328 (M, 4), 207 (M−CH_2PhOCH_3, 17), 121 (CH_2PhOCH_3, 100). ^1H NMR ($CDCl_3$): δ [ppm] = 1.62 (s, 3H, $NCH_2CH{=}C(CH3)_2$), 1.67–1.75 (m, 1H, 4-H), 1.70 (s, 3H, $NCH_2CH{=}C(CH_3)_2$), 1.83–1.91 (m, 1H, 4-H), 2.27 (ddd, J = 13.3/8.6/1.6 Hz, 1H, 3-H), 2.77 (dd, J = 10.2/2.3 Hz, 1H, piperazine-H), 2.83–2.93 (m, 3H, piperazine-H), 3.00–3.04 (m, 1H, piperazine-H), 3.05–3.17 (m, 2H, NCH_2CH=C(CH_3)$_2$), 3.17–3.19 (m, 1H, piperazine-H), 3.25 (ddd,

J = 13.3/10.2/8.6 Hz, 1H, 3-H), 3.60 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.64 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.79 (s, 3H, ArOCH_3), 5.15–5.21 (m, 1H, NCH$_2$CH=C(CH$_3$)$_2$), 6.83 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.19 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2912 (m, $\nu_{\text{C–H aliph.}}$), 1708 (s, $\nu_{\text{C=O ketone}}$), 1611 (m)/1510 (s, $\nu_{\text{C=C arom.}}$), 1441 (m, $\delta_{\text{C-H aliph.}}$), 1244 (s)/1033 (m, $\nu_{\text{C–O}}$), 820 (m, $\Gamma_{\text{p-subst. arom.}}$).

6.15. (+)-(1*S*,5*R*)-8-(4-Methoxybenzyl)-6-(3-methylbut-2-en-1-yl)-6,8-diazabicyclo[3.2.2]nonan-2-one (ent-12)

As described for the preparation of **12**, the enantiomer ent-**11** (78 mg, 0.19 mmol) was reacted with $LiAlH_4$ (0.78 mL of a 1.0 M solution in THF, 0.78 mmol) in THF (30 mL) and afterwards hydrolyzed with 1.0 M HCl (5 mL) to give ent-**12** as a yellow oil, yield 42 mg (66%). $C_{20}H_{28}N_2O_2$ (328.5). Purity by HPLC: method 2a: t_R = 19.8 min, purity 97.2%; method 1: t_R = 18.2 min, purity 99.5%. $[\alpha]_D^{20}$ = +76.1 (c = 0.16; CH_2Cl_2).

6.16. (−)-(1*S*,5*S*)-6-Benzyl-2,2-dimethoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (13)

Under N_2 a solution of **10** (26 mg, 0.08 mmol) and tetrabutylammonium iodide (6 mg, 0.02 mmol) in THF (20 mL) was cooled to −78 °C. Then a 2.0 M solution of sodium hexamethyldisilazane in THF (0.05 mL, 0.09 mmol) was added dropwise. After stirring the mixture at −78 °C for 40 min, benzyl bromide (0.05 mL, 66 mg, 0.39 mmol) was added. The mixture was stirred at −78 °C for 1 h and was then allowed to warm to rt. After stirring the mixture at rt for 2 h, water was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, 5 mL, R_f = 0.12) to give **13** as a colorless oil, yield 22 mg (67%). $C_{24}H_{28}N_2O_5$ (424.5). Purity by HPLC: method 1: t_R = 20.2 min, purity 97.5%. $[\alpha]_D^{20}$ = −21.1 (c = 0.42; CH_2Cl_2). MS (EI): m/z [%] = 424 (M, 3), 121 (CH_2PhOCH_3, 29), 101 ($H_2CCHC(OCH_3)_2$, 100), 91 (CH_2Ph, 13). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.44–1.54 (m, 1H, 3-H), 1.70–1.80 (m, 1H, 3-H), 1.87–1.99 (m, 2H, 4-H), 3.17 (s, 3H, OCH_3), 3.28 (s, 3H, OCH_3), 3.79 (s, 3H, ArOCH_3), 3.88 (dd, J = 5.5/1.6 Hz, 1H, 5-H), 3.94 (d, J = 14.9 Hz, 1H, NCH_2Ar), 4.05 (s, 1H, 1-H), 4.51 (d, J = 14.9 Hz, 1H, NCH_2Ph), 4.59 (d, J = 14.9 Hz, 1H, NCH_2Ph), 5.18 (d, J = 14.9 Hz, 1H, NCH_2Ar), 6.86 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.12 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.20–7.24 (m, 2H, NCH$_2C_6H5$), 7.27–7.35 (m, 3H, NCH$_2C_6H_5$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3029 (w, $\nu_{\text{C–H arom.}}$), 2942 (m, $\nu_{\text{C–H aliph.}}$), 1676 (s, $\nu_{\text{C=O amide}}$), 1612 (m)/1512 (m, $\nu_{\text{C=C arom.}}$), 1451 (m, $\delta_{\text{C-H aliph.}}$), 1245 (m)/1036 (m, $\nu_{\text{C-O}}$), 808 (w, $\Gamma_{\text{p-subst. arom.}}$), 732 (m)/700 (m, $\Gamma_{\text{mono-subst. arom.}}$).

6.17. (+)-(1*R*,5*R*)-6-Benzyl-2,2-dimethoxy-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (ent-13)

As described for the preparation of **13**, the enantiomer ent-**10** (30 mg, 0.09 mmol), tetrabutylammonium iodide (7 mg, 0.02 mmol), 2 M solution of sodium hexamethyldisilazane in THF (0.05 mL, 0.10 mmol) and benzyl bromide (0.05 mL, 77 mg, 0.45 mmol) were reacted in THF (20 mL) to give ent-**13** as a colorless oil, yield 18 mg (47%). $C_{24}H_{28}N_2O_5$ (424.5). Purity by HPLC: method 1: t_R = 20.4 min, purity 96.3%. $[\alpha]_D^{20}$ = +20.4 (c = 0.33; CH_2Cl_2).

6.18. (−)-(1*R*,5*S*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonan-2-one (14)

Under N_2 a 1.0 M solution of $LiAlH_4$ (0.76 mL, 0.76 mmol) was added to an ice-cooled solution of **13** (80 mg, 0.19 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then 1.0 M HCl (5 mL) was added under ice-cooling and the mixture was stirred at 0 °C for 10 min and then refluxed for 3 h. After cooling down the mixture was alkalized with a saturated aqueous solution of $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, CH_2Cl_2/methanol = 50/1, V = 5 mL, R_f = 0.39) to give **14** as a colorless oil, yield 33 mg (*R*,*S*)-**107** (50%). $C_{22}H_{26}N_2O_2$ (350.5). Purity by HPLC: method 2a: t_R = 23.2 min, purity 99.6%; method 1: t_R = 18.3 min, purity 99.5%. $[\alpha]_D^{20}$ = −61.5 (c = 0.85; CH_2Cl_2). MS (EI): m/z [%] = 350 (M, 3), 229 (M–CH_2PhOCH_3, 3), 121 (CH_2PhOCH_3, 100), 91 (CH_2Ph, 36). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.63–1.73 (m, 1H, 4-H), 1.76–1.85 (m, 1H, 4-H), 2.31 (ddd, J = 13.3/8.6/1.6 Hz, 1H, 3-H), 2.82 (dd, J = 10.2/2.3 Hz, 1H, piperazine-H), 2.86–2.92 (m, 3H, piperazine-H), 3.03–3.07 (m, 1H, piperazine-H), 3.20 (t, J = 3.1 Hz, 1H, piperazine-H), 3.28 (ddd, J = 13.3/10.2/8.6 Hz, 1H, 3-H), 3.61 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.66 (d, J = 12.5 Hz, 2H, NCH_2Ar), 3.74 (d, J = 12.5 Hz, 1H, NCH_2Ar), 3.79 (s, 3H, ArOCH_3), 6.83 (d, J = 8.6 Hz, 2H, 3′-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.18 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.20–7.25 (m, 1H, NCH$_2C_6H5$), 7.26–7.30 (m, 4H, NCH$_2C_6H_5$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3027 (w, $\nu_{\text{C–H arom.}}$), 2913 (m, $\nu_{\text{C–H aliph.}}$), 1708 (s, $\nu_{\text{C=O ketone}}$), 1610 (m)/1510 (s, $\nu_{\text{C=C arom.}}$), 1453 (m, $\delta_{\text{C-H aliph.}}$), 1244 (m)/1030 (m, $\nu_{\text{C–O}}$), 819 (w, $\Gamma_{\text{p-subst. arom.}}$), 698 (m, $\Gamma_{\text{mono-subst. arom.}}$).

6.19. (+)-(1*S*,5*R*)-6-Benzyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonan-2-one (ent-14)

As described for the preparation of **14**, the enantiomer ent-**13** (118 mg, 0.28 mmol) was reacted with $LiAlH_4$ (1.11 mL of a 1.0 M solution in THF, 1.11 mmol) in THF (40 mL) and afterwards hydrolyzed with 1.0 M HCl (10 mL) to give ent-**14** as a colorless oil, yield 20 mg (21%). $C_{22}H_{26}N_2O_2$ (350.5). Purity by HPLC: method 2a: t_R = 23.8 min, purity 99.6%; method 1: t_R = 18.5 min, purity 99.0%. $[\alpha]_D^{20}$ = +64.1 (c = 0.44; CH_2Cl_2).

6.20. (−)-(1S,5S)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-phenyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (15)

Under N_2 a mixture of **10** (50 mg, 0.15 mmol), iodobenzene (0.34 mL, 610 mg, 2.99 mmol), K_2CO_3 (21 mg, 0.15 mmol) and CuI (3 mg, 0.015 mmol) in DMF (20 mL) was heated at 150 °C for 72 h. After the mixture was cooled to rt, water was added and the mixture was extracted with diethyl ether (6×). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, cyclohexane/ethyl acetate = 2/1, V = 5 mL, R_f = 0.16) to give **15** as a yellow oil, yield 44 mg (72%). $C_{23}H_{26}N_2O_5$ (410.5). Purity by HPLC: method 1: t_R = 20.2 min, purity 100%. $[\alpha]_D^{20}$ = −46.2 (c = 0.18; CH_2Cl_2). MS (EI): m/z [%] = 410 (M, 5), 121 (CH_2PhOCH_3, 32), 101 ($H_2CCHC(OCH_3)_2$, 100). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.86–1.96 (m, 1H, 3-H), 1.99–2.09 (m, 1H, 3-H), 2.13–2.23 (m, 2H, 4-H), 3.21 (s, 3H, OCH_3), 3.32 (s, 3H, OCH_3), 3.78 (s, 3H, ArOCH_3), 4.06 (d, J = 14.9 Hz, 1H, NCH_2Ar), 4.11 (s, 1H, 1-H), 4.40 (dd, J = 5.5/1.6 Hz, 1H, 5-H), 5.24 (d, J = 14.9 Hz, 1H, NCH_2Ar), 6.86 (d, J = 8.6 Hz, 2H, 3'-H$_{4\text{-methoxybenzyl}}$, 5′-H$_{4\text{-methoxybenzyl}}$), 7.18 (d, J = 8.6 Hz, 2H, 2′-H$_{4\text{-methoxybenzyl}}$, 6′-H$_{4\text{-methoxybenzyl}}$), 7.23–7.27 (m, 1H, 4′-H$_{\text{phenyl}}$), 7.28 (d, J = 7.8 Hz, 2H, 2′-H$_{\text{phenyl}}$, 6′-H$_{\text{phenyl}}$), 7.39 (t, J = 7.8 Hz, 2H, 3′-H$_{\text{phenyl}}$, 5′-H$_{\text{phenyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2946 (m, $\nu_{\text{C–H aliph.}}$), 1682 (s, $\nu_{\text{C=O amide}}$), 1612 (m)/1512 (m, $\nu_{\text{C=C arom.}}$), 1456 (m, $\delta_{\text{C-H aliph.}}$), 1246 (m)/1032 (m, $\nu_{\text{C–O}}$), 808 (w, $\Gamma_{\text{p-subst. arom.}}$), 729 (m, $\Gamma_{\text{mono-subst. arom.}}$).

6.21. (+)-(1R,5R)-2,2-Dimethoxy-8-(4-methoxybenzyl)-6-phenyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (ent-15)

As described for the preparation of **15**, the enantiomer ent-**10** (70 mg, 0.21 mmol) was reacted with iodobenzene (0.47 mL, 854 mg, 4.19 mmol), K_2CO_3 (29 mg, 0.21 mmol) and CuI (4 mg, 0.021 mmol) in DMF (20 mL) to give ent-**15** as a yellow oil, yield 73 mg (85%). $C_{23}H_{26}N_2O_5$ (410.5). Purity by HPLC: method 1: t_R = 20.0 min, purity 98.6%. $[\alpha]_D^{20}$ = +48.4 (c = 0.32; CH_2Cl_2).

6.22. (+)-(1R,5S)-8-(4-Methoxybenzyl)-6-phenyl-6,8-diazabicyclo[3.2.2]nonan-2-one (16)

Under N_2 a 1.0 M solution of $LiAlH_4$ (0.58 mL, 0.58 mmol) was added to an ice-cooled solution of **15** (60 mg, 0.15 mmol) in THF (20 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then 1.0 M HCl (5 mL) was added under ice-cooling and the mixture was stirred at 0 °C for 10 min and then refluxed for 3 h. After cooling down the mixture was alkalized with a saturated aqueous solution of $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, CH_2Cl_2, V = 5 mL, R_f = 0.26) to give **16** as a yellow oil, yield 10 mg (20%). $C_{21}H_{24}N_2O_2$ (336.4). Purity by HPLC: method 2a: t_R = 21.6 min, purity 98.7%; method 1: t_R = 17.8 min, purity 98.3%. $[\alpha]_D^{20}$ = +34.7 (c = 0.41; CH_2Cl_2). MS (EI): m/z [%] = 336 (M, 12), 308 (M–CO, 16), 215 (M–CH_2PhOCH_3, 6), 121 (CH_2PhOCH_3, 100), 77 (Ph, 27). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.99–2.13 (m, 2H, 4-H), 2.29 (ddd, J = 13.3/7.0/3.1 Hz, 1H, 3-H), 2.95–3.00 (m, 1H, 9-H), 3.19–3.23 (m, 1H, 9-H), 3.27–3.31 (m, 1H, 7-H), 3.40 (dt, J = 13.3/9.4 Hz, 1H, 3-H), 3.46–3.48 (m, 1H, 1-H), 3.56 (dd, J = 11.0/2.3 Hz, 1H, 7-H), 3.73 (s, 2H, NCH_2Ar), 3.80 (s, 3H, ArOCH_3), 4.10–4.14 (m, 1H, 5-H), 6.63 (d, J = 7.8 Hz, 2H, 2′-H_{phenyl}, 6′-H_{phenyl}), 6.72 (t, J = 7.8 Hz, 1H, 4′-H_{phenyl}), 6.86 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.20–7.26 (m, 4H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$, 3′-$H_{phenyl}$, 5′-$H_{phenyl}$). ^{13}C NMR ($CDCl_3$): δ [ppm] = 28.4 (1C, C-4), 37.3 (1C, C-3), 42.2 (1C, C-7), 53.6 (1C, C-5), 54.6 (1C, C-9), 55.5 (1C, ArOCH_3), 60.1 (1C, NCH_2Ar), 67.9 (1C, C-1), 111.2 (2C, C-2′$_{phenyl}$, C-6′$_{phenyl}$), 114.1 (2C, C-3′$_{4\text{-methoxybenzyl}}$, C-5′$_{4\text{-methoxybenzyl}}$), 117.0 (1C, C-4′$_{phenyl}$), 129.6 (2C, C-3′$_{phenyl}$, C-5′$_{phenyl}$), 129.9 (2C, C-2′$_{4\text{-methoxybenzyl}}$, C-6′$_{4\text{-methoxybenzyl}}$), 130.3 (1C, C-1′$_{4\text{-methoxybenzyl}}$), 148.2 (1C, C-1′$_{phenyl}$), 159.2 (1C, C-4′$_{4\text{-methoxybenzyl}}$), 215.2 (1C, 2-C). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3037 (w, $\nu_{C-H\ arom.}$), 2932 (m, $\nu_{C-H\ aliph.}$), 1703 (s, $\nu_{C=O\ ketone}$), 1598 (s)/1511 (s, $\nu_{C=C\ arom.}$), 1463 (m, $\delta_{C-H\ aliph.}$), 1240 (m)/1029 (m, ν_{C-O}), 836 (w, $\Gamma_{p\text{-subst. arom.}}$), 753 (m, $\Gamma_{mono\text{-subst. arom.}}$).

6.23. (−)-(1S,5R)-8-(4-Methoxybenzyl)-6-phenyl-6,8-diazabi cyclo[3.2.2]nonan-2-one (ent-16)

As described for the preparation of **16**, the enantiomer ent-**15** (70 mg, 0.17 mmol) was reacted with $LiAlH_4$ (0.68 mL of a 1.0 M solution in THF, 0.68 mmol) in THF (20 mL) and afterwards hydrolyzed with 1.0 M HCl (5 mL) to give ent-**16** as a yellow oil, yield 43 mg (75%). $C_{21}H_{24}N_2O_2$ (336.4). Purity by HPLC: method 2a: t_R = 21.4 min, purity 98.4%; method 1: t_R = 17.7 min, purity 99.1%. $[\alpha]_D^{20}$ = −36.2 (c = 0.35; CH_2Cl_2).

6.24. (−)-(1R,5S)-8-(4-Methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonan-2-one (17)

Under N_2 a 1.0 M solution of $LiAlH_4$ (1.30 mL, 1.30 mmol) was added to an ice-cooled solution of **10** (109 mg, 0.33 mmol) in THF (30 mL). The mixture was stirred at 0 °C for 10 min and then heated to reflux for 16 h. Then 1.0 M HCl (10 mL) was added under ice-cooling and the mixture was stirred at 0 °C for 10 min and then refluxed for 3 h. After cooling down the mixture was alkalized with a saturated aqueous solution of $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by fc (Ø = 2 cm, h = 15 cm, CH_2Cl_2/methanol = 9.5/0.5, V = 10 mL, R_f = 0.08) to give 17 as a pale yellow oil, yield 50 mg (59%). $C_{15}H_{20}N_2O_2$ (260.3). $[\alpha]_D^{20}$ = −28.2 (c = 0.41; CH_2Cl_2). MS (EI): m/z [%] = 260 (M, 3), 232 (M–CO, 9), 121 (CH_2PhOCH_3, 100). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.57–1.67 (m, 1H, 4-H), 1.96–2.04 (m, 1H, 4-H), 2.32 (ddd, J = 13.3/7.8/1.6 Hz, 1H, 3-H), 2.90–2.96 (m, 2H, 7-H, 9-H), 3.02 (dd, J = 11.0/2.3 Hz, 1H, 9-H), 3.19–3.21 (m, 1H, 1-H), 3.25–3.33 (m, 2H, 3-H, 7-H), 3.47–3.51 (m, 1H, 5-H), 3.62 (d, J = 13.3 Hz, 1H, NCH_2Ar), 3.69 (d, J = 13.3 Hz, 1H, NCH_2Ar), 3.79 (s, 3H, ArOCH_3), 6.83 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.19 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$). The signal for the proton of the NH group could not be detected. ^{13}C NMR ($CDCl_3$): δ [ppm] = 31.0 (1C, C-4), 37.6 (1C, C-3), 40.8 (1C, C-7), 49.8 (1C, C-5), 53.5 (1C, C-9), 55.5 (1C, ArOCH_3), 60.1 (1C, NCH_2Ar), 68.2 (1C, C-1), 114.0 (2C, C-3′$_{4\text{-methoxybenzyl}}$, C-5′$_{4\text{-methoxybenzyl}}$), 129.9 (2C, C-2′$_{4\text{-methoxybenzyl}}$, C-6′$_{4\text{-methoxybenzyl}}$), 130.6 (1C, C-1′$_{4\text{-methoxybenzyl}}$), 159.0 (1C, C-4′$_{4\text{-methoxybenzyl}}$), 216.9 (1C, 2-C). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3341 (m br, ν_{N-H}), 2922 (s, $\nu_{C-H\ aliph.}$), 1705 (s, $\nu_{C=O\ ketone}$), 1610 (s)/1510 (s, $\nu_{C=C\ arom.}$), 1442 (m, $\delta_{C-H\ aliph.}$), 1242 (s)/1031 (m, ν_{C-O}), 815 (m, $\Gamma_{p\text{-subst. arom.}}$).

6.25. (+)-(1S,5R)-8-(4-Methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonan-2-one (ent-17)

As described for the preparation of **17**, the enantiomer ent-**10** (70 mg, 0.21 mmol) was reacted with $LiAlH_4$ (0.84 mL of a 1.0 M solution in THF, 0.84 mmol) in THF (30 mL) and afterwards hydrolyzed with 1.0 M HCl (5 mL) to give ent-**17** as a pale yellow oil, yield 21 mg (39%). $C_{15}H_{20}N_2O_2$ (260.3). $[\alpha]_D^{20}$ = +26.9 (c = 0.11; CH_2Cl_2).

6.26. (−)-(1R,5S)-6-Benzoyl-8-(4-methoxybenzyl)-6,8-diazabicyclo[3.2.2]nonan-2-one (18)

Under N_2 **17** (35 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (20 mL). Under ice-cooling triethylamine (0.02 mL, 14 mg, 0.13 mmol) and benzoyl chloride (0.03 mL, 38 mg, 0.27 mmol) were added and the mixture was stirred at rt for 16 h. Then a saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by fc (Ø = 1 cm, h = 15 cm, CH_2Cl_2/methanol = 100/1, V = 5 mL, R_f = 0.13) to give **18** as a pale yellow oil, yield 30 mg (61%). $C_{22}H_{24}N_2O_3$ (364.5). Purity by HPLC: method 2b: t_R = 14.0 min, purity 95.5%; method 1: t_R = 14.8 min, purity 96.2%. $[\alpha]_D^{20}$ = −77.8 (c = 0.22; CH_2Cl_2). MS (EI): m/z [%] = 364 (M, 59), 336 (M–CO, 21), 121 (CH_2PhOCH_3, 100), 105 (PhCO, 35). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.85–1.92 (m, 2× 0.3H, 4-H^b), 1.99––2.08 (m, 0.7H, 4-H^a), 2.13–2.23 (m, 0.7H, 4-H^a), 2.25–2.32 (m, 0.7H, 3-H^a), 2.36–2.44 (m, 0.3H, 3-H^b), 2.94–3.09 (m, 1× 0.7H + 3× 0.3H, 9-H^a, 3-H^b (1× 0.3H), 9-H^b (2× 0.3H)), 3.24–3.29 (m, 2× 0.7H, 1-H^a, 9-H^a (1× 0.7H)), 3.34–3.49 (m, 2× 0.7H + 1× 0.3H, 3-H^a (1× 0.7H), 7-H^a (1× 0.7H), 1-H^b), 3.62–3.69 (m, 1H + 1× 0.7H, NCH_2Ar, 7-H^a), 3.70–3.81 (m, 1H + 1× 0.3H, NCH_2Ar, 7-H^b), 3.79 (s, 3H, ArOCH_3), 3.84–3.89 (m, 0.3H, 7-H^b), 4.07–4.12 (m, 0.3H, 5-H^b), 4.84–4.87 (m, 0.7H, 5-H^a), 6.84 (d, J = 8.6 Hz, 2H, 3′-$H_{4\text{-methoxybenzyl}}$, 5′-$H_{4\text{-methoxybenzyl}}$), 7.18 (d, J = 8.6 Hz, 2H, 2′-$H_{4\text{-methoxybenzyl}}$, 6′-$H_{4\text{-methoxybenzyl}}$), 7.32–7.42 (m, 5H, NCO$C_6H_5$). Two rotamers exist in the ratio 70: 30 (a: major rotamer; b: minor rotamer). ^{13}C NMR ($CDCl_3$): δ [ppm] = 24.3 (1C, C-4^a), 27.3 (1C, C-4^b), 34.6 (1C, C-3^b), 35.3 (1C, C-3^a), 39.9 (1C, C-7^b), 41.7 (1C, C-7^a), 47.9 (1C, C-5^a), 50.7 (1C, C-5^b), 51.4 (1C, C-9^a), 52.0 (1C, C-9^b), 53.4 (1C, ArOCH_3^{a,b}), 57.8 (1C, NCH_2Ar^{a,b}), 63.5 (1C, C-1^b), 65.1 (1C, C-1^a),

112.1 (2C, C-$3^{a,b\prime}_{4\text{-methoxybenzyl}}$, C-$5^{a,b\prime}_{4\text{-methoxybenzyl}}$), 124.4 (2C, C-$2^{b\prime}_{benzoyl}$, C-$6^{b\prime}_{benzoyl}$), 124.9 (2C, C-$2^{a\prime}_{benzoyl}$, C-$6^{a\prime}_{benzoyl}$), 126.6 (2C, C-$3^{a\prime}_{benzoyl}$, C-$5^{a\prime}_{benzoyl}$), 126.8 (2C, C-$3^{b\prime}_{benzoyl}$, C-$5^{b\prime}_{benzoyl}$), 127.6(1C, C-$1^{a,b\prime}_{4\text{-methoxybenzyl}}$), 127.8 (2C, C-$2^{a,b\prime}_{4\text{-methoxybenzyl}}$, C-$6^{a,b\prime}_{4\text{-methoxybenzyl}}$), 127.9 (1C, C-$4^{b\prime}_{benzoyl}$), 128.0 (1C, C-$4^{a\prime}_{benzoyl}$), 133.6 (1C, C-$1^{a,b\prime}_{benzoyl}$), 157.1 (1C, C-$4^{a,b\prime}_{4\text{-methoxybenzyl}}$), 168.5 (1C, $NCOC_6H_5{}^{a,b}$), 212.2 (1C, C-$2^{a,b}$). Two rotamers exist in the ratio 70: 30 (a: major rotamer; b: minor rotamer). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2933 (m, $\nu_{C-H\ aliph.}$), 1710 (s, $\nu_{C=O\ ketone}$), 1628 (s, $\nu_{C=O\ amide}$), 1511 (s, $\nu_{C=C\ arom.}$), 1446 (m, $\delta_{C-H\ aliph.}$), 1244 (s)/1030 (m, ν_{C-O}), 818 (m, $\Gamma_{p\text{-subst. arom.}}$), 702 (m, $\Gamma_{mono\text{-subst. arom.}}$).

6.27. (+)-(1S,5R)-6-Benzoyl-8-(4-methoxybenzyl)-6,8-diazabi cyclo[3.2.2]nonan-2-one

(ent-18)

As described for the preparation of **18**, the enantiomer ent-**17** (23 mg, 0.09 mmol) was reacted with benzoyl chloride (0.01 mL, 12 mg, 0.18 mmol) and triethylamine (0.01 mL, 9 mg, 0.09 mmol) in CH_2Cl_2 (20 mL) to give ent-**18** as a pale yellow oil, yield 15 mg (47%). $C_{22}H_{24}N_2O_3$ (364.5). Purity by HPLC: method 2b: t_R = 14.6 min, purity 96.4%; method 1: t_R = 14.8 min, purity 96.9%. $[\alpha]_D^{20}$ = +74.5 (c = 0.22; CH_2Cl_2).[****]

7. Receptor binding studies

7.1. Materials and general procedures

The guinea pig brains and rat livers were commercially available (Harlan-Winkelmann, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo-Finnigan). Filter: Printed Filtermat Typ B (Perkin–Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin–Elmer). The scintillation analysis was performed using Meltilex (Typ A) solid scintillator (Perkin–Elmer). The solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidification of the scintillator at rt, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin–Elmer). The counting efficiency was 20%.

7.2. Membrane preparation for the σ_1 assay (modified according to Refs. 11,12)

Five guinea pig brains were homogenized with the potter (500–800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200 *g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500*g* for 20 min at 4 °C. The pellet was resuspended in 5–6 volumes of buffer (50 mM Tris, pH 7.4) and centrifuged again at 23,500 *g* (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5–6 volumes of buffer, the protein concentration was determined according to the method of Bradford[15] by using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

7.3. Performing of the σ_1 assay (modified according to Refs. 11,12)

The assay was performed with the radioligand [^{3}H]-(+) pentazocine (42.5 Ci/mmol; Perkin–Elmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM Tris, pH 7.4) in a total volume of 200 μL for 180 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled (+) pentazocine. The K_d-value of the radioligand [^{3}H]-(+)-pentazocine is 2.9 nM.[16]

7.4. Membrane preparation for the σ_2 assay (modified according to Refs. 11,12)

Two rat livers were cut into smaller pieces and homogenized with a potter (500–800 rpm, 10 up-and-down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000*g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31,000*g* for 20 min at 4 °C. The final pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford[15] by using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 2 mg protein/mL.

7.5. Performing of the σ_2 assay (modified according to Refs. 11,12)

The test was performed with the radioligand [^{3}H]di-*o*-tolylguanidine (50 Ci/mmol; ARC). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-di-*o*-tolylguanidine, 500 nM (+)-pentazocine, and buffer (50 mM Tris, pH 8.0) in a total volume of 200 μL for 180 min at rt. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After each well was washed five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled di-*o*-tolylguanidine. The K_d-value of the radioligand [^{3}H]-di-*o*-tolylguanidine is 17.9 nM.[17]

7.6. NMDA assay

The preparation of the receptor material and the assay were performed according to a literature procedure.[12]

7.7. Data analysis

All experiments were carried out in triplicates using standard 96-well multiplates (Diagonal). The IC_{50}-values were determined in competition experiments with six concentrations of the test compounds and were calculated with the program GraphPad Prism 3.0 (GraphPad Software) by nonlinear regression analysis. The K_i-values were calculated according to Cheng and Prusoff.[18] The K_i-values are given as the mean value ± SEM from three independent experiments.

8. Cytotoxicity assay

All cell lines were obtained from the German Collection of Microbiology and Cell Culture (DSZK, Braunschweig, FRG). Cytotoxicity

testing was done by using a microtiter assay based on staining cells with crystal violet as described in detail in Ref. 14 To determine the IC_{50} values, five serially diluted stock solutions of test compound in DMF were used in the studies; concentrations giving *T*/*C* values between 10% and 90% were used to estimate the IC_{50} values, which were calculated by least-squares analysis of the dose-response curves.

Acknowledgements

This work was performed within the International Research Training Group 'Complex Functional Systems in Chemistry: Design, Synthesis and Applications' in collaboration with the University of Nagoya. Financial support of this IRTG by the *Deutsche Forschungsgemeinschaft* is gratefully acknowledged.

References and notes

1. Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; De Costa, B.; Rice, K. C. *Pharmacol. Rev.* **1990**, *42*, 355.
2. Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. *Trends Pharmacol. Sci.* **1992**, *13*, 85.
3. Bowen, W. D. *Pharm. Acta Helv.* **2000**, *74*, 211.
4. Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H.; Striessnig, J.; Kempner, E.; Glossman, H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8072.
5. Aydar, E.; Palmer, C. P.; Klyachko, V. A.; Jackson, M. B. *Neuron* **2002**, *34*, 399.
6. Aydar, E.; Palmer, C. P.; Djamgoz, M. B. *Cancer Res.* **2004**, *64*, 5029.
7. Glennon, R. A.; Ablordeppey, S. Y.; Ismaiel, A. M.; El-Ashmawy, M. B.; Fischer, J. B.; Howie, K. B. *J. Med. Chem.* **1994**, *37*, 1214.
8. Holl, R.; Schepmann, D.; Grünert, R.; Bednarski, P. J.; Wünsch, B. *Bioorg. Med. Chem*. **2009**, *17*, 777.
9. Jung, B.; Englberger, W.; Wünsch, B. *Arch. Pharm. Pharm. Med. Chem.* **2005**, *338*, 281.
10. Renaud, J.; Bischoff, S. F.; Buhl, T.; Floersheim, P.; Fournier, B.; Geiser, M.; Halleux, C.; Kallen, J.; Keller, H.; Ramage, P. *J. Med. Chem.* **2005**, *48*, 364.
11. Maier, C. A.; Wünsch, B. *J. Med. Chem.* **2002**, *45*, 438.
12. Wirt, U.; Schepmann, D.; Wünsch, B. *Eur. J. Org. Chem.* **2007**, 462.
13. Colabufo, N. A.; Berardi, F.; Contino, M.; Niso, M.; Abate, C.; Perrone, R.; Tortorella, V. *Naunyn Schmiedebergs Arch. Pharmacol.* **2004**, *370*, 106.
14. Bracht, K.; Boubakari; Grunert, R.; Bednarski, P. J. *Anticancer Drugs* **2006**, *17*, 41.
15. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
16. DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1992**, *227*, 371.
17. Mach, R. H.; Smith, C. R.; Childers, S. R. *Life Sci.* **1995**, *57*, PL57.
18. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.