Eur. J. Med. Chem. 36 (2001) 375–388

PII: S0223-5234(01)01222-3/SCO

**Laboratory Note**

# Syntheses and evaluation of halogenated cytisine derivatives and of bioisosteric thiocytisine as potent and selective nAChR ligands

Peter Imming[a], Paul Klaperski[a], Milton T. Stubbs[a], Gunther Seitz[a]*, Daniela Gündisch[b]

[a]Department of Pharmaceutical Chemistry, Philipps-University, Marbacher Weg 6, D-35032 Marburg, Germany
[b]Department of Pharmaceutical Chemistry, Rheinische Friedrich-Wilhelms-University, Kreuzbergweg 26, D-53115 Bonn, Germany

Received 17 October 2000; revised 19 January 2001; accepted 1 February 2001

**Abstract** – We have developed one-step syntheses of halogenated derivatives of (−)-cytisine featuring a halogen substituent at positions 3, 5 or 3 and 5 of the 2-pyridone fragment, and prepared the novel bioisosteric thiocytisine by oxygen–sulphur exchange. The affinities of these pyridone-modified analogs of (−)-cytisine for $(\alpha4)_2(\beta2)_3$ and α7* nAChRs in rat forebrain membranes were determined by competition with (±)-[$^3$H]epibatidine and [$^3$H]MLA, respectively. The 3-halocytisines **7** possess subnanomolar affinities for $(\alpha4)_2(\beta2)_3$ nAChRs, higher than those found for (−)-cytisine as well as for the 5-halocytisines **8** and 3,5-dihalocytisines **6**. In contrast to the parent alkaloid the 3-halogenated species display much a higher affinity for the α7* nAChR subtype. The most potent molecule was 3-bromocytisine (**7b**) with preferential selectivity (200-fold) for the $(\alpha4)_2(\beta2)_3$ subtype [$K_i = 10$ pM (α4β2) and 2.0 nM (α7*)]. Replacement of the lactam with a thiolactam pharmacophore to thiocytisine (**12**) resulted in a subnanomolar affinity for the $(\alpha4)_2(\beta2)_3$ nAChR subtype ($K_i = 0.832$ nM), but in a drastic decrease of affinity for the α7* subtype; thiocytisine (**12**) has a $K_i$ value of 4000 nM (α7*), giving a selectivity of 4800-fold for the neuronal $(\alpha4)_2(\beta2)_3$-nAChR and thus displaying the best affinity–selectivity profile in the series under consideration. © 2001 Éditions scientifiques et médicales Elsevier SAS

**halocytisines / thiocytisines / nAChR subtype selectivity / bioisosterism**

## 1. Introduction

There is accumulating evidence that ligands acting with high agonistic affinity at nicotinic acetylcholine receptors (nAChRs) may possibly be utilised as therapeutics [1–18] in the treatment of various neurological and mental disorders related to a decrease in cholinergic function: these include, e.g. senile dementia of the Alzheimer type, Parkinson's disease, attention deficit hyperactivity disorder, Tourette's syndrome, depression and ulcerative colitis, in addition to nicotine addiction, tardive dyskinesia and schizophrenia. Moreover, worldwide interest in nAChR agonists as potential analgesics has emerged, representing an attractive area of research in pain control [1, 2, 4, 6, 11, 14]. Although several promising ligands for nAChRs have been developed [1–18] during the past few years, there is still a need for potent agents that would interact more selectively with the neuronal nicotinic receptors and display no or minimal side-effects as compared with naturally occurring prototypical agonists for nAChRs such as (−)-nicotine (**1**) or (−)-epibatidine (**2**) [1, 4, 10, 18], whose therapeutic usefulness is limited by several untoward effects. (see *figure 1.*)

1,2,3,4,5,6-Hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-8-one, (−)-cytisine (**3**) [19, 20] with numbering through the text, easily accessible by extracting seeds from *Laburnum anagyroides medicus* (Fabaceae) [21], showed a remarkable combination of properties,

*Abbreviations:* nAChR, nicotinic acetylcholine receptor; MLA, methyllycaconitine; SAR, structure–activity relationship; PET, positron emission tomography; DMAP, 4-dimethylaminopyridine; BTX, bungarotoxine; HEPES, N-(2-hydroxyethyl)-piperazine-N′-(2-ethanesulphonic acid); HSS, HEPES–salt solution; HRMS, high-resolution mass spectrometry.

* Corresponding author
*E-mail address:* seitzg@mailer.uni-marburg.de (G. Seitz).

sharing various physiological effects with (−)-nicotine (**1**) [22]. It is acutely toxic and its toxicological responses include nausea, convulsions, and death by respiratory failure. In comparison with (−)-nicotine (**1**), however, (−)-cytisine (**3**) is a more potent nAChR ligand, displaying higher selectivity toward the $(\alpha4)_2(\beta2)_3$ nAChR subtype combined with subnanomolar affinity [22–25]. Advantageously the *Laburnum*-alkaloid shows a longer half-life in vivo than (−)-nicotine [26], crosses the blood–brain-barrier [18] and exhibits comparable efficacy and potency to (−)-nicotine in stimulating dopamine release from striatal synaptosomes [18].

Until now, only a few structure–activity relationship (SAR) studies on (−)-cytisine derivatives have been reported [18, 27]. Thus, in our effort to gain novel nAChR agonists [28, 29] with possibly improved pharmacodynamic profiles and safety over the natural alkaloids **1**, **2** and **3**, we decided to synthesise a number of structurally modified derivatives of (−)-cytisine. Replacement of a hydrogen atom by a halogen in one or more specific positions of a biologically active lead compound may substantially improve the intrinsic biological activity [15, 30, 31]. Thus, we investigated the influence of halogen substituents such as chlorine, bromine and iodine on the in vitro affinity for $(\alpha4)_2(\beta2)_3$ and α7* nAChRs, in order to gain further insight into the SAR of these species.

In addition we were interested in the divalent bioisosteric replacement [32] of the lactam pharmacophore, the hydrogen bond acceptor functionality of which is required for maintaining biological activity [4, 23]. Since bioisosteric replacement of a lactam by a thiolactam pharmacophore has been used extensively

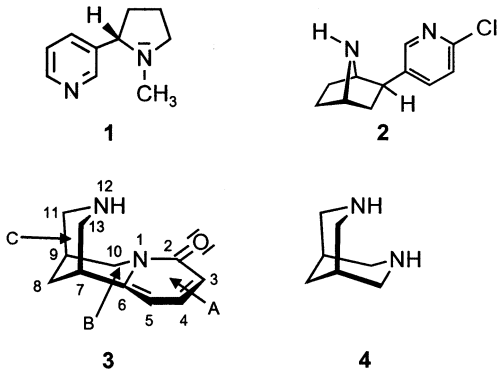



**Figure 1.** Natural ligands for nAChRs and the bispidine core of (−)-cytisine (**3**).

in medicinal chemistry, retaining activity both in vitro and in vivo [32], we anticipated that an oxygen–sulphur exchange in the pharmacophoric lactam moiety might lead to a retention of activity or even enhanced potency associated with higher selectivity. This would reveal whether the size of the substituents and the presence of hydrogen bond acceptors were important factors for the affinity of the nAChR ligand. Such compounds might also possess reduced toxicity.

A recent paper describing the synthesis of analogs of (−)-cytisine for in vivo studies of nAChRs using PET [33] (positron emission tomography) and several patents [18, 34] claiming (−)-cytisine (**3**) and its derivatives as useful agents for the treatment of neurogenerative diseases and their use in addiction therapy prompted us to report our own independently obtained results.

In this communication we describe one-step preparations of several halogenated cytisine derivatives and the first synthesis of the previously unknown thiocytisine, as well as the in vitro binding affinities of the target molecules for $(\alpha4)_2(\beta2)_3$ and α7* nAChRs, determined by competition with (±)-[$^3$H]epibatidine [35] and [$^3$H]MLA, respectively [36].

## 2. Chemistry

(−)-Cytisine (**3**) is a chiral quinolizidine alkaloid with a tricyclic skeleton, consisting of the A-, B-, and C-rings. It is characterised by a bispidine framework **4** fused to a 2-pyridone moiety. The constitution of **3** has been elucidated by chemical degradation [37] and by syntheses [38–42]; the absolute configuration of the two chiral centers was established by Okuda et al. to be 7*R*,9*S* [37]. The solution and crystal structure of the alkaloid **3** has been studied using NMR techniques and X-ray crystallography, respectively [43]. The 2-pyridone moiety of **3** allows various electrophilic substitution reactions [19, 33, 34]. These properties inspired us to prepare primarily the halogenated cytisines **6–8** (compounds with the **a** designation are chloro derivatives, whereas the **b** and **c** designations denote, respectively, bromo and iodo compounds).

Although a variety of synthetic approaches to halocytisines has been developed [19, 33, 34, 44], most of the previously known halogenation reactions of the alkaloid **3** afforded three-step procedures (including N-12 protecting and deprotecting besides the halo-

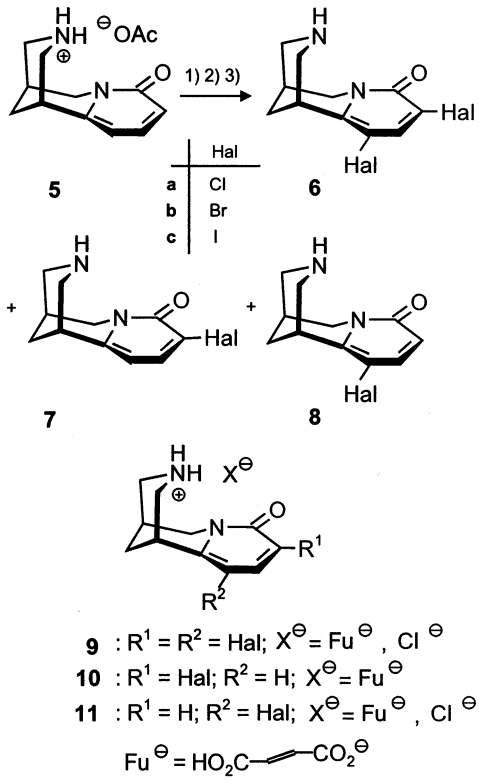



**Figure 2.** Reagents and conditions: (1) Hal = Cl; *N*-chlorosuccinimide (1 equiv. mol) in $AcOH/H_2O = 3:2$, 1 h reflux, column chromatography (silica gel, $CHCl_3/CH_3OH = 10:1$); fraction 1, **6a** (5%); fraction 2, **8a** (40%); fraction 3, **7a** (26%). (2) Hal = Br; *N*-bromosuccinimide (1 equiv. mol) in $AcOH/H_2O = 3:2$, 2 h reflux, column chromatography (silica gel, $CHCl_3/CH_3OH = 10:1$); fraction 1, **6b** (5%); fraction 2, **8b** (27%); fraction 3, **7b** (27%). (3) Hal = I; iodine chloride (1 equiv. mol) in AcOH (100%), 16 h r.t., column chromatography (silica gel, $CHCl_3/CH_3OH = 10:1$); fraction 1, **6c** (1%); fraction 2, mixture **7c**, **8c** (51%); fraction 3, **8c** (10%).

genation step). Thus, we employed a technique that proved effective for a one-step procedure. The general route for the cytisine derivatives is illustrated in *figure 2.* The key step in the one-step syntheses of the halocytisines **6-8** was the halogenation reaction of the N-12 protonated (−)-cytisinium acetate (**5**), prepared in situ by employing aqueous acetic acid (60%) as the solvent and utilising *N*-chloro-, *N*-bromosuccinimide and iodine chloride (ICl) as halogen transfer reagents. Using a manifold excess of the halogenating agent, a twofold substitution of the 3- and 5- positions of the 2-pyridone moiety occurred, leading nearly quantitatively to the halocytisines of type **6**. However, when only one molar equivalent of the halogen transfer reagent was employed, a mixture of three halogenated products was obtained with the monosubstituted species **7** and **8** predominating. The mixture of the three halocytisines of types **6**, **7** and **8** could be easily separated by careful flash chromatography on silica gel, and converted subsequently into the crystalline hydrogen fumaric salts **9–11.** The structures of the crystalline salts **9–11** were conclusively confirmed by mass, $^1H$ and $^{13}C$ NMR spectral analysis (see Section 6).

Since bioisosterism serves as a valuable aid in SAR studies and new drug design [32], we were interested in the previously unknown bioisosteric thioanalog of (−)-cytisine herein named thiocytisine. It was anticipated that (−)-cytisine (**3**) might easily be converted to the corresponding thioanalog by treatment with convenient sulphur transfer reagents. Because the well known thionating agent tetraphosphorus decasulphide (for a recent review see Ref. [45]) involved a long reaction time combined with low yields, we utilised a novel, microwave-accelerated, only recently published method [46]. In this process (−)-cytisine (**3**) was simply mixed with Lawesson's reagent (0.5 equiv.) and then irradiated under solvent free conditions, to obtain the desired (−)-thiocytisine (**12**) with nearly 40% yield.

Detailed spectroscopic data analysis (MS, IR, UV, $^1H$ and $^{13}C$ NMR) conclusively proved the expected structure of **12**. The mass spectrum of **12** shows three signals in the region of the molecular ion ($m/z = 208$, $M^+ + 2$, 207, $M^+ + 1$, and 206, $M^+$), a typical pattern of a compound containing one sulphur atom. Substitution of sulphur for oxygen shifted the UV absorbance maximum from $\lambda_{max} = 320$ nm for (−)-cytisine (**3**) to $\lambda_{max} = 357$ nm for (−)-thiocytisine (**12**). The IR spectrum of **12** in the solid state (KBr) exhibited a strong absorption band ($\nu$ C=S) at 1206 $cm^{-1}$, providing evidence for the presence of the thiocarbonyl group [47–50], while the lactam **3** displayed only comparatively weak absorptions in this region. (A similar strong absorption is also found in the acetylated thiocytisine (**13**).) (See *figures 3 and 4.*)

The $^1H$ and $^{13}C$ NMR spectra of solutions of (−)-thiocytisine (**12**) in $CHCl_3$-$d_1$, taken at 400 and 100.5 MHz, respectively are similar to those of the parent alkaloid [43] (see Section 6). The thiocarbonyl carbon resonance was observed at $\delta = 179.82$, assigned on the basis of its chemical shift. Eventually, appropriate crystals for an X-ray crystallographic analysis were obtained, which conclusively established

the constitution of compound **12** and revealed the intact bispidine structure annulated with a 2-thiopyridone moiety.

*Figure 4* shows the molecular structure of **12** (with the atomic numbering used) and the conformation that (−)-thiocytisine (**12**) adopts in the solid state. The A ring of **12**, as in (−)-cytisine (**3**) [43], is planar, and the bond length and angles give evidence of a considerable delocalisation of electrons on the ring. Ring B exhibits a half-chair, and ring C a rigid chair conformation with C-7, C-9, C-11, and C-13 lying in a plane. The C(2)=S bond length of 1.684(5) Å is much longer (0.074 Å) than that in thioketones, (with a value of 1.61 Å as a reasonable baseline [50, 51]) due to the resonance in the thiolactam group. Because C=S bonds appear to rely on the (+)-*M*-influence of the substituents to generate a $\delta^{(-)}$ charge on S, it is this resonance that increases the charge density on the thiocarbonyl sulphur and makes hydrogen bond formation possible (normally the sulphur atom in a thione group is a much weaker hydrogen bond acceptor than the oxygen atom of a C=O group) [51].

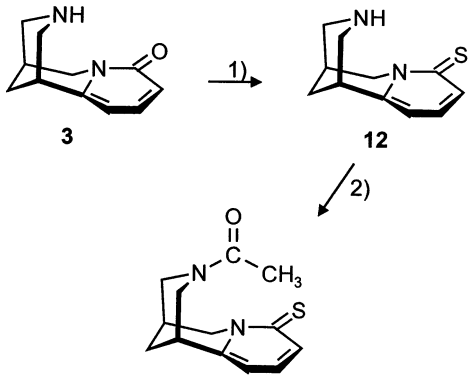



**Figure 3.** Reagents and conditions: (1) Lawesson's reagent, microwave irradiation (800 W), alumina column chromatography, 37%. (2) DMAP, $Na_2CO_3$, $CH_3COCl$; 67%.

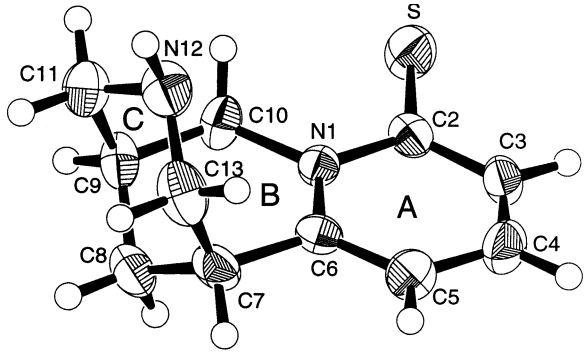



**Figure 4.** ortep diagram (50% probability ellipsoids) showing the solid state conformation of **12**. The hydrogen atoms are drawn as spheres with arbitrary radii.

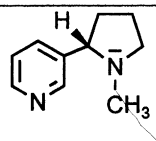
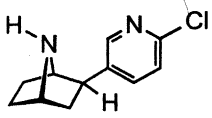
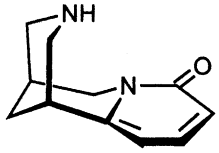
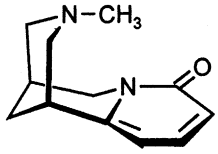
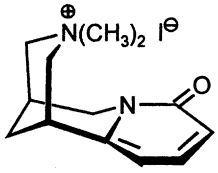
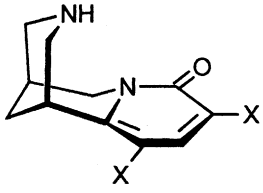
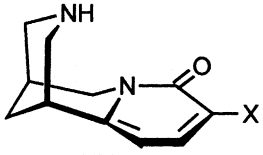
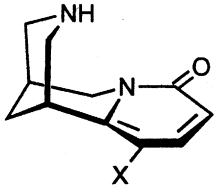
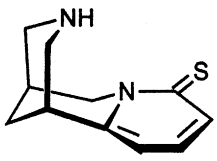
Acetylation of the secondary amine **3** with an excess of acetyl chloride/DMAP was successful in providing the acetylated derivative **13** (as a pair of rotamers about the N–C=O bond). The $^1$H NMR spectrum of **13** was recognised as a series of doubled signals, stemming from two rotamers [52].

## 3. Pharmacology

The cytisine derivatives listed in *table I* were tested for their in vitro affinity for $(\alpha4)_2(\beta2)_3$ and α7* nAChRs subtypes by radioligand binding assays. To determine the affinities for the $(\alpha4)_2(\beta2)_3$ nAChR subtype a previously described competition assay was used with (±)-[$^3$H]epibatidine and the P2 membrane fraction of Sprague–Dawley rat forebrain. These studies demonstrated that the specific binding of (±)-[$^3$H]epibatidine to crude synaptic membranes of rat forebrain, at concentrations up to 800 pM, is characterised by a single population of binding sites with $K_d = 8 \pm 0.3$ pM [35]. It has been previously found that the predominant receptor with high affinity for [$^3$H]nicotine, (−)-[$^3$H]cytisine, (±)-[$^3$H]epibatidine, and 5-[$^{125}$I]iodo-A-85380 in rat brain is composed of α4 and β2 subunits [31]. As shown in *table I*, competition assays yielded $K_i$ values of (−)-cytisine for the $(\alpha4)_2(\beta2)_3$ nAChR subtype that are consistent with recently reported in vitro measurements of the natural alkaloid [35] using the same assay conditions.

To characterise the binding of each of the (−)-cytisine variants to the α7* nAChR subtype, [$^3$H]-MLA and membrane fractions isolated from the rat brain were used. [$^3$H]MLA bound to a single population of binding sites exhibited a $K_d$ value of 1.2±0.2 nM ($n = 3$). The affinity determined was in good agreement with previously published values [36] ($K_d = 1.86$ nM). [$^3$H]MLA binds to rat brain membranes with a regional distribution characteristic of α-BTX-sensitive, putative α7* subunit-containing nAChRs [36]. The parent compound (−)-cytisine showed a $K_i$ value of 261 nM using [$^3$H]MLA. This pattern agrees with previous findings [31] where $K_i$ values (260±20 nM) for (−)-cytisine were determined using[$^{125}$I]α-BTX.

**Table I.** Radioligand binding affinities of several cytisine variants to $(\alpha4)_2(\beta2)_3$ and α7* nAChRs in comparison with (−)-nicotine, (±)-epibatidine and (−)-cytisine.

| Structure | Compound | $(\alpha4)_2(\beta2)_3$ [$^3$H]epibatidine rat brain $K_i$(nM) | α7* [$^3$H]MLA rat brain $K_i$(nM) |
|---|---|---|---|
|  | (-)-Nicotine | 0.838 ± 0.132 [31] | 130 ± 10 [$^{125}$I] α-BTX [31] |
|  | (±)-Epibatidine | 0.008 ± 0.001 [31], 0.0072 ± 0.0005 [36] | 4 ± 0.5 [$^{125}$I] α-BTX [31] |
|  | (-)-Cytisine | 0.122 ± 0.01, 0.124 ± 0.014 [36] | 261 ± 24 260 ± 20 [$^{125}$I]α-BTX [31] |
|  | N-Methyl-cytisine (caulophylline) | 5.7 ± 1.5 | 15,000 |
|  | N,N-Dimethyl-cytisinium-iodide (caulophylline-methiodide) | 0.238 ± 0.046 | 1,100 |
|  | 3,5-Dichloro-cytisine | 2.5 ± 0.4 | 1,000 |
| | 3,5-Dibromo-cytisine | 10.8 ± 0.4 | 1,500 |
| | 3,5-Diiodo-cytisine | 0.520 ± 0.015 | 41 ± 2 |
|  | 3-Chlorocytisine | 0.022 ± 0.005 | 2.5 ± 0.3 |
| | 3-Bromocytisine | 0.010 ± 0.001 | 2.0 ± 0.3 |
| | 3-Iodocytisine | 0.017 ± 0.002 | 1.5 ± 0.1 |
|  | 5-Chlorocytisine | 2.5 ± 0.3 | 1,000 |
| | 5-Bromocytisine | 0.308 ± 0.014 | 28 ± 2 |
| | 5-Iodocytisine | 0.230 ± 0.02 | 21 ± 2 |
|  | Thiocytisine | 0.832 ± 0.056 | 4,000 |

As shown in *table I*, competition assays yielded $K_i$ values of 0.838 nM for (−)-nicotine (**1**) and 0.008 nM for (±)-epibatidine (**2**). These results are consistent with recently reported in vitro measurements of the natural alkaloids [15].

## 4. Results and discussion

We have demonstrated that halogenation of the 2-pyridone moiety of (−)-cytisine (**3**) can be carried out successfully in a one-step approach utilising (−)-cytisinium acetate (**5**), in situ prepared in aqueous acetic acid (60%) as the starting material. The syntheses of the twofold substituted halocytisines of type **6** were accomplished with yields better than 80%, employing a twofold excess of the halogenating agent mentioned. The same method proved useful in the preparation of the 3-halocytisines **7** and the 5-halocytisines **8** utilising one molar equivalent of the halogen transfer reagent. The resulting mixture of the halocytisines **6**, **7** and **8** could be separated by simple chromatography on silica gel. The ratio of the 3- and 5- regioisomers was shown to be dependent on the halogen transfer reagent used (ratios of isolated yield of **6/7/8**: **a**, 5:40:26%; **b**, 5:27:27%; **c**, 1:35:19%). An efficient comparison of our results with the literature data proved difficult because detailed information concerning the yields obtained with the three-step procedures (including an additional chromatographic separation) in the patent [34] and the short communication [33] were not available in all cases. Nevertheless the one-step procedure described herein, in our opinion, seemed to be advantageous.

Compared with (±)-epibatidine (**2**), (−)-cytisine (**3**) exhibited a 15-fold lower affinity ($K_i = 0.122$ nM) for the $(\alpha4)_2(\beta2)_3$ subtype and a 60-fold lower affinity for the α7* subtype ($K_i = 261$ nM). *N*-methylation of **3** to caulophylline caused a dramatic loss in affinity dropping into the nanomolar range ($K_i = 5.7$ nM) for $(\alpha4)_2(\beta2)_3$ nAChRs, whereas the dimethylated salt, caulophylline *methiodide* disclosed only a twofold lower affinity ($K_i = 0.238$ nM) in comparison with the parent alkaloid.

The introduction of halogen substituents in most cases resulted in a retention of affinity. The 3-halocytisines retained subnanomolar affinity ($K_i$ values of 0.01–0.022 nM) to central $(\alpha4)_2(\beta2)_3$-nAChRS (Br> I>Cl>H); the bromo representative of this series **7b** showed even an about one order of magnitude higher affinity than the parent alkaloid **3** ($K_i = 0.122$ nM), similar to (±)-epibatidine (**2**) ($K_i = 0.008$ nM). The halogen substituents at position 5 of the 2-pyridone moiety exerted an evidently smaller impact on the binding affinity, with $K_i$ values dropping in the higher picomolar (Br) and even nanomolar ranges (Cl). Twofold halogenation in the 3,5-position of the 2-pyridone moiety significantly reduced the affinity. Thus, the emerging trend, that the presence of a bulkier substituent at position 5 of the 2-pyridone pharmacophore reduces the affinity for the $(\alpha4)_2(\beta2)_3$ nAChR subtype, is corroborated with the 3,5-dihalocytisines **6a–c** [$K_i$ values range from 0.520 (I) to 10.8 nM (Br)].

The affinities of caulophylline methiodide and caulophylline for the $(\alpha4)_2(\beta2)_3$ receptor exceeded their affinities for the α7* subtype much more than that observed with the parent (−)-cytisine (**3**). Remarkably, the 3-halocytisines exhibited the highest affinity for the α7* subtype in this series, comparable with the affinities of α-BTX and MLA. Halogenation in position 5 or twofold halogenation in 3 and 5 positions resulted in a decrease of affinity for the α7* subtype compared with those of the 3-halocytisines.

The divalent bioisosteric replacement of the lactam oxygen in **3** by sulphur yielded (−)-thiocytisine (**12**) characterised by a thiolactam pharmacophore. Though somewhat less potent, the thiolactam **12** showed a sevenfold lower affinity compared with the parent alkaloid with subnanomolar binding affinity for the $(\alpha4)_2(\beta2)_3$ receptor ($K_i = 0.832$ nM). Remarkably, the novel (−)-thiocytisine shows the best affinity–selectivity profile for $(\alpha4)_2(\beta2)_3$ nAChRs in this series with an affinity for the α7* subtype $K_i =$ 4000 nM. The observed differences in affinity of (−)-cytisine (**3**) and (−)-thiocytisine (**12**) agree well with the Sheridan model [53] of the nicotinic pharmacophore, suggesting that the optimal distances (a–b = 4.7 Å; a–c = 4.0 Å; b–c = 1.2 Å) between three pharmacophoric elements are most important for the three-dimensional arrangement also for (−)-cytisine (**3**) and its derivatives, recognised by the nAChRs:

1. a cationic center [e.g. the basic or quaternised N-12 atom of ring C of (−)-cytisine (**3**)]
2. an electronegative atom capable of accepting a hydrogen bond (e.g. the lactam carbonyl oxygen of **3**)
3. a dummy point or an atom to define a line along which the hydrogen bond may form (in the case of **3** the lactam carbonyl carbon).

A detailed analysis of the structure and conformation of (−)-cytisine (**3**) in the solution and in the solid

state [43] revealed that the alkaloid featured similar spatial arrangements of the pharmacophoric elements (a) and (b) to (−)-nicotine (**1**): the N-12–O-2 distance of 4.89 Å agrees well with the current nicotinic pharmacophor model (calculated internitrogen distance in **1**: 4.87 Å) [53]. As expected, the divalent bioisosteric replacement of the lactam by the thiolactam pharmacophore changes the three-dimensional arrangement of the ligand [32]. The probable reasons for the lower affinity of (−)-thiocytisine (**12**) are, firstly, the different a–b distance (N-12–S-2) of 5.248 Å (surprisingly similar to the calculated internitrogen distance in (±)-epibatidine (**2**)) and the a–c distance (N-12–C-2) of 4.290 Å, taken from the X-ray data of **12** (see Section 6), and secondly, the C=O/C=S exchange resulting in a modification of the hydrogen-bond acceptor ability, crucial for nAChR affinity [23, 53]. As already discussed the effective electronegativity of S in thiolactams is increased by conjugative interactions between C=S and the lone pair of the vicinal N substituent, a clear example of resonance-induced hydrogen bonding at sulphur acceptors [51]. Comparing the frequencies of hydrogen-bond formation of the C=S and C=O acceptors (e.g. (−)-cytisine (**3**) and (−)-thiocytisine (**12**)) the C=S···HN hydrogen bonds are anticipated to be significantly weaker than their C=O···HN analogs, thus accounting for the lower $(\alpha4)_2(\beta2)_3$ affinity of **12** and the dramatic loss of affinity for the α7* nAChR subtype.

## 5. Conclusions

In our continuing efforts to develop new ligands with high affinity and better selectivity for the multivarious subtypes of nAChRs, we have prepared and evaluated several analogs of the highly toxic alkaloid (−)-cytisine (**3**). We found that several of these (−)-cytisine-based compounds with halogen atoms as the substituents at position 3, 5, or 3 and 5 of the 2-pyridone fragment or characterised by a bioisosteric thiolactam pharmacophore instead of the lactam functionality proved to be highly potent nAChR ligands. Most of these species possess subnanomolar affinity for the $(\alpha4)_2(\beta2)_3$ nAChR subtype in membranes from rat forebrain. In this series, 3-bromocytisine (**7b**) shows the highest affinity for $(\alpha4)_2(\beta2)_3$ nAChR subtype ($K_i = 10$ pM), exceeding that of the parent alkaloid (−)-cytisine (**3**) by approximately one order of magnitude and comparable to the affinity of (±)-epibatidine (**2**). In contrast, (−)-cytisine derivatives of type **8** with a 5-halo-substituted 2-pyridone ring exhibit a substantially lower affinity similar to the corresponding 3,5-dihalocytisines **6**. The thiolactam **12** was found to retain much of the biological activity of the parent alkaloid with a subnanomolar binding affinity for the $(\alpha4)_2(\beta2)_3$ nAChR subtype. For the α7* nAChR subtype affinities, the 3-halocytisines proved the most active species in the series under consideration, comparable with the affinities of α-BTX and MLA. Bioisosteric C=O/C=S replacement resulted in a drastic decrease of affinity for the α7* subtype: thus, the novel thiocytisine (**12**) displays the best affinity–selectivity profile for $(\alpha4)_2(\beta2)_3$ nAChRs in this series. It would be very interesting to find out in future studies what kind of substitution in the (−)-cytisine template would be able to change the selectivity toward the α7* receptor.

## 6. Experimental protocols

### 6.1. General chemistry

Standard vacuum techniques were used in the handling of the air-sensitive materials. Melting points were determined on a 'Leitz-Heiztischmikroskop' HM-Lux and are uncorrected. Solvents were dried and freshly distilled before use according to literature procedures. IR spectra were recorded on a Perkin–Elmer 257, 398 and a Nicolet FT-IR spectrometer 510-P; liquids were run as films, solids as KBr pellets. $^1$H NMR and $^{13}$C NMR were recorded on a JEOL JNM-GX 400 and LA 500 and $\delta$ values are given in ppm relative to tetramethylsilane as internal standard ($J$ values in Hz). Mass spectra were measured with a Fisons Instruments VG 70-70 E spectrometer at a 70 eV ionising voltage (EI). Column chromatography was carried out on Merck silica gel 40 (40–60 mesh, flash chromatography) or Merck silica 60, 70–230 mesh. Reactions were monitored by thin-layer chromatography (TLC) by using plates of silica gel (0.063–0.200 mm, Merck) or silicagel-60$F_{254}$ microcards (Riedel de Haen). Optical rotations were determined on a Jasco DIP-370 polarimeter. UV–vis spectra were recorded on a Shimadzu scanning spectrophotometer UV-2101 PC. Combustion analyses were performed internally. Microwave irradiation was carried out in a domestic oven (800 W) privileg 8017.

### 6.2. Synthetic procedures

#### 6.2.1. (−)-3,5-Dichlorocytisine (9,11-dichloro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido-[1,2-a](1,5-)diazocin-8-one) (**6a**) [33, 34, 44]

To a stirred solution of **3** (190.2 mg, 1.00 mmol) in aqueous acetic acid (10 mL, 60%) was added dropwise at reflux temperature a solution of *N*-chlorosuccinimide (280.2 mg, 2.10 mmol) in aqueous acetic acid (10 mL, 60%) within 10 min. After stirring for 1.5 h at reflux temperature the solvent was removed in vacuo at 70°C (bath temperature). The residue was treated with aqueous NaOH (10 mL, 10%) and the resulting aqueous solution extracted four times with $CHCl_3$ (10 mL). The combined organic phase was dried over anhydrous $MgSO_4$ (3 g), filtered, and the solvent removed in vacuo at 45°C (bath temperature), to give 304 mg of a viscous yellow oil. The crude material was purified by silica gel chromatography (18.5 cm×3 cm, $CHCl_3/CH_3OH = 5:1$) to yield **6a** (249.8 mg, 83%) as pale yellow, viscous oil, $R_f = 0.38$ (silica gel, $CHCl_3/CH_3OH = 5:1$). $[\alpha]_D^{20} = -38.6$ ($c = 0.3$, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) 3364 (NH), 1648 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 213 nm (3.96), 244 (3.75), 332 (3.85). $^1H$ NMR ($CHCl_3$-$d_1$, 400 MHz): $\delta = 1.91$ (m, 1H, 8-$H_\alpha$), 2.00 (m, 2H, 8-$H_\beta$, NH), 2.38 (s, broad, 1H, 9-H), 2.96 (dd, $^2J = 11.9$ Hz, $^3J = 2.2$ Hz, 1H, 11-$H_\alpha$), 3.00 (d, $^2J = 12.3$ Hz, 1H, 13-$H_\alpha$), 3.10 (d, $^2J = 12.3$ Hz, 1H, 13-$H_\beta$), 3.16 (d, $^2J = 11.9$ Hz, 1H, 11-$H_\beta$), 3.40 (s, broad, 1H, 7-H), 3.97 (dd, $^2J = 15.7$ Hz, $^3J = 5.7$ Hz, 1H, 10-$H_\alpha$), 4.16 (d, $^2J = 15.7$ Hz, 1H, 10-$H_\beta$), 7.57 (s, 1H, 4-H). (For the $^1H$ NMR (250 MHz) see Ref. [34]. $^{13}C$ NMR ($CHCl_3$-$d_1$, 100.5 MHz): $\delta$ = 26.0 (C-8), 27.2 (C-9), 32.3 (C-7), 50.1 (C-10), 51.8 (C-11)[1], 52.5 (C-13)[1], 108.9 (C-5), 122.6 (C-3), 137.8 (C-4), 145.9 (C-6), 158.5 (C-2). MS (70 eV, 130°C): $m/z$(%) = 260 (20, $M^+ + 2$), 259 (5, $M^+ + 1$), 258 (35, $M^+$), 44 (100). HRMS. Found: 258.0280. Calc. for $C_{11}H_{12}Cl_2N_2O$: 258.0326.

#### 6.2.2. Chlorination of (−)-cytisine **3** with 1 equiv. mol. of *N*-chlorosuccinimide to **6a**, **7a** and **8a**

To a stirred solution of **3** (570.9 mg, 3.00 mmol) in aqueous acetic acid (10 mL, 60%) was added dropwise at reflux temperature a solution of *N*-chlorosuccinimide (401.0 mg, 3.00 mmol) in aqueous acetic acid (10 mL, 60%) within 8 min. After stirring for 1 h at reflux temperature the solvent was removed in vacuo at 70°C (bath temperature) to give 1517 mg of a yellowish, viscous oil. The crude material was purified by silica gel chromatography (21×3 cm, $CHCl_3$–$CH_3OH$ = 10:1), obtaining three fractions. Isolated yields were as follows: fraction 1 **(6a)**, colourless, viscous oil, 35.6 mg (5%), fraction 2 (**7a**), yellowish viscous oil, 270.0 mg (40%), fraction 3 **(8a),** yellow crystalline powder, 175.8 mg (26%).

[1] Assignment not confirmed.

#### 6.2.3. (−)-3-Chlorocytisine (9-chloro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1.2-a][1.5]-diazocin-8-one) fumarate (**10a**)

Using the conditions employed for the preparation of **11a, 7a** (92.7 mg, 0.41 mmol) was treated with fumaric acid (48.5 mg, 0.41 mmol) to yield the salt **10a** (114.3 mg, 81%), colourless crystals, m.p. 196–198°C. $[\alpha]_D^{20} = -81.5$ (c = 0.2, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) 3428 (NH), 1660 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$ = 211 nm (4.17), 236 (3.84), 320 (3.91). $^1H$ NMR (DMSO-$d_6$, 400 MHz): $\delta = 1.87$ (m, 1H, 8-$H_\alpha$), 1.90 (m, 1H, 8-$H_\beta$), 2.37 (s, broad, 1H, 9-H), 2.91 (dd, $^2J = 11.9$ Hz, $^3J = 6.2$ Hz, 1H, 11-$H_\alpha$), 2.93 (dd, $^2J = 11.7$ Hz, $^3J = 6.0$ Hz, 1H, 13-$H_\alpha$), 2.99 (d, $^2J = 11.7$ Hz, 1H, 13-$H_\beta$), 3.05 (m, 2H, 11-$H_\beta$, 7-H), 3.80 (dd, $^2J = 15.4$ Hz, $^3J = 6.6$ Hz, 1H, 10-$H_\alpha$), 3.94 (d, $^2J = 15.4$ Hz, 1H, 10-$H_\beta$), 6.11 (d, $^3J = 7.7$ Hz, 1H, 5-H), 6.54 (s, 2H, vinyl-H), 7.62 (d, $^3J = 7.5$ Hz, 1H, 4′-H). $^{13}C$ NMR (DMSO-$d_6$, 125.8 MHz): $\delta = 24.5$ (C-8), 26.0 (C-9), 33.3 (C-7), 49.9 (C-10), 50.5 (C-11), 51.5 (C-13), 103.7 (C-5), 120.1 (C-3), 134.2 (2 vinyl-C), 136.9 (C-4), 149.8 (C-6), 158.3 (C-2), 166.5 (2 carboxylate-C). Anal. Found: C, 52.74; H, 5.04; N, 8.19. Calc. for $C_{15}H_{17}ClN_2O_5$ (341.37): C, 52.87; H, 5.03; N, 8.22%.

#### 6.2.4. (−)-5-Chlorocytisine (11-chloro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1.2-a][1.5]-diazocin-8-one) fumarate (**11a**)

To a stirred solution of compound **8a** (131.2 mg, 0.58 mmol) in 2-propanol (2 mL) was added at reflux temperature fumaric acid (68.1 mg, 0.58 mmol) in 2-propanol (3 mL). The stirred solution was kept at 22°C for 4 h, the precipitated solid was collected, washed three times with anhydrous diethyl ether (5 mL) and dried over silica in vacuo, yielding colourless crystals (174.7 mg, 88%), m.p. 188–190°C. $[\alpha]_D^{20} = -36.5$ ($c$ = 0.1, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) 3444 (NH), 1667 (CO), 1654 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 209 nm (4.27), 239 (4.00), 322 (3.80). $^1H$ NMR (DMSO-$d_6$, 400 MHz): $\delta = 1.85$ (m, 1H, 8-$H_\alpha$), 1.92 (m, 1H, 8-$H_\beta$), 2.35 (s, broad, 1H, 9-H), 2.88 (dd, $^2J = 11.8$ Hz, $^3J = 1.8$ Hz, 1H, 11-$H_\alpha$), 2.91 (dd, $^2J = 12.2$ Hz, $^3J = 2.5$ Hz, IH,

13-$H_\alpha$), 3.01 (m, 2H, 13-$H_\beta$, 11-$H_\beta$), 3.31 (s, broad, 1H, 7-H), 3.74 (dd, $^2J = 15.7$ Hz, $^3J = 6.6$ Hz, 1H, 10-$H_\alpha$), 3.85 (d, $^2J = 15.4$ Hz, 1H, 10-$H_\beta$), 6.29 (d, $^3J = 9.5$ Hz, 1H, 3-H), 6.56 (s, 2H, vinyl-H), 7.43 (d, $^3J = 9.7$ Hz, 1H, 4-H). $^{13}$C NMR (DMSO-$d_6$, 125.8 MHz): $\delta = 24.8$ (C-8), 25.9 (C-9), 31.2 (C-7), 48.5 (C-10), 49.8 (C-11), 50.7 (C-13), 108.3 (C-5), 116.6 (C-3), 134.0 (2 vinyl-C), 139.4 (C-4), 146.8 (C-6), 160.9 (C-2), 166.3 (2 carboxylate-C). Anal. Found: C, 52.82; H, 5.18; N, 8.12. Calc. for $C_{15}H_{17}ClN_2O_5$ (341.37): C, 52.87; H, 5.03; N, 8.22%.

### *6.2.5. (−)-3,5-Dibromocytisine (9,11-dibromo-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido-[1.2-a][1.5]diazocin-8-one) (**6b**) [33, 34, 44, 54]*

To a stirred solution of **3** (190.2 mg, 1.00 mmol) in acetic acid (100%, 25 mL) was added dropwise at room temperature a solution of bromine (0.20 mL, 622 mg, 3.91 mmol) in acetic acid (100%, 3 mL). After stirring for 3 h ice water (100 mL) was added and the mixture stirred vigorously for 30 min. The resulting suspension was filtered, and the collected solid washed twice with diethyl ether (10 mL). After drying the orange solid (394 mg) in vacuo it was suspended in a mixture of ethanol (6 mL) and water (4 mL). The stirred suspension was heated to reflux for 5 min and the resulting solution cooled to ca. 5°C. The crystalline precipitate was collected, washed twice with diethyl ether (10 mL) and dried in vacuo over $P_4O_{10}$ to give the hydrobromide of **6b** as colourless needles, m.p. 220–226°C (dec.) Lit. [54]: m.p. 238°C. (−)-3,5-Dibromocytisine-hydrobromide (179 mg, 0.42 mmol) was dissolved in $CH_2Cl_2$ (10 mL). To the solution was added water (20 mL) and aqueous sodium hydroxide (2 mL, 10%). After vigorous stirring of the two-phases-system, $CH_2Cl_2$ (10 mL) was added, the aqueous layer separated, and extracted three times with $CH_2Cl_2$ (30 mL). The combined organic phases were dried over $Na_2SO_4$ (3 g), filtered, and the solvent was removed in vacuo. The crude material was purified by silica gel chromatography (10 cm × 2 cm, $CHCl_3/CH_3OH = 5:1$) to yield **6b** (134 mg, 92%), which appeared to be a colourless solid, m.p. 108–110°C (Lit. [54]: m.p. 67–75°C). $[\alpha]_D^{20}$ (of the corresponding fumarate salt) = −23.1(0.2, MeOH); IR (KBr): $\nu$ (cm$^{-1}$) 3319 (NH), 1642 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 222 nm (3.97), 244 (3.82), 334 (3.96). $^1$H NMR ($CHCl_3$-$d_1$, 400 MHz): $\delta = 1.80$ (s, broad, 1H, NH), 1.93 (m, 1H, 8-$H_\alpha$), 1.97 (m, 1H, 8-$H_\beta$), 2.35 (m, 1H, 9-H), 2.93* (dd, $^2J = 12.0$ Hz, $^3J = 2.2$ Hz, 1H, 11-$H_\alpha$)[1], 2.99 (d, $^2J = 12.0$ Hz, 1H, 11-$H_\beta$), 3.09 (d, $^2J = 11.9$ Hz, 1H, 13-$H_\beta$), 3.18* (d, $^2J = 11.9$ Hz, 1H, 13-$H_\alpha$), 3.34 (m, 1H, 7-H), 3.96 (dd, $^2J = 15.6$ Hz, $^3J = 6.7$ Hz, 1H, 10-$H_\alpha$), 4.14 (d, $^2J = 15.6$ Hz, 1H, 10-$H_\beta$), 7.87 (s, 1H, 4-H). *Assignment not confirmed. For the $^1$H NMR spectrum of the corresponding hydrochloride salt see Ref. [34]. $^{13}$C NMR ($CHCl_3$-$d_1$, 100.5 MHz): $\delta = 26.1$ (C-8), 27.3 (C-9), 34.7 (C-7), 50.1 (C-10), 52.2 (C-13), 52.5 (C-11), 97.3 (C-5), 112.51 (C-3), 143.6 (C-4), 147.9 (C-6), 158.7 (C-2). MS (70 eV, 160°C): $m/z$ (%) = 350 (46, $^{81}$Br, $^{81}$Br−M$^+$), 349 (13, $^{79}$Br, $^{81}$Br−M$^+$+1), 348 (91, $^{79}$Br, $^{81}$Br−M$^+$), 347 (8, $^{79}$Br, $^{79}$Br−M$^+$+1), 346 (47, $^{79}$Br, $^{79}$Br−M$^+$), 305 (100). Anal. Found: C, 37.90; H, 3.63; N, 8.01. Calc. for $C_{11}H_{12}Br_2N_2O$ (348.0): C, 37.96; H, 3.47; N, 8.05%.

### *6.2.6. Bromination of (−)-cytisine **3** with 1 equiv. mol. of N-bromosuccinimide to **6b**, **7b** and **8b***

To a stirred solution of **3** (951 mg, 5.00 mmol) in aqueous acetic acid (20 mL, 60%) was added dropwise at reflux temperature a solution of *N*-bromosuccinimide (890.4 mg, 5.00 mmol) in aqueous acetic acid (30 mL, 60%) within 15 min. After stirring for 2 h at reflux temperature the solvent was removed in vacuo at 75°C (bath temperature) to yield a brown, viscous oil (2960 mg), which was purified by silica gel chromatography (20 × 3 cm, $CHCl_3$–$CH_3OH$ = 10:1), obtaining three fractions. The isolated yields were as follows: fraction 1 (**6b**), 90.8 mg (5%), colourless solid, m.p. 108–110°C, fraction 2 (**7b**), 362.3 mg (27%), colourless solid, m.p. 51–53°C, fraction 3 **(8b),** 364.9 mg (27%), pale yellow solid, m.p. 103–106°C.

### *6.2.7. (−)-3-Bromocytisine (9-bromo-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1.2-a][1.5]-diazocin-8-one) (**7b**)*

$[\alpha]_D^{20} = -83.3$ ($c = 0.2$, $CH_3OH$); IR (KBr): $\nu$ (cm$^{-1}$) 3429 (NH), 1641 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 210 nm (3.94), 239 (3.63), 323 (3.90). $^1$H NMR ($CHCl_3$-$d_1$, 400 MHz,): $\delta = 1.75$ (s, broad, 1H, NH), 1.95 (m, 2H, 8-$H_\alpha$, 8-$H_\beta$), 2.35 (s, broad, 1H, 9-H), 2.92 (s, broad, 1H, 7-H), 3.00 (d, $^2J = 12.2$ Hz, 1H, 11-$H_\alpha$), 3.05 (d, $^2J = 11.2$ Hz, 1H, 13-13-$H_\alpha$), 3.07 (d, $^2J = 11.2$ Hz, 1H, H-13$_\beta$), 3.11 (d, $^2J = 12.2$ Hz, 1H, 11-$H_\beta$), 3.95 (dd, $^2J = 15.6$ Hz, $^3J = 6.6$ Hz, 1H, 10-$H_\alpha$), 4.18 (d, $^2J = 15.6$ Hz, 1H, 10-$H_\beta$), 5.93 (d, $^3J = 7.6$ Hz, 1H, 5-H), 7.68 (d, $^3J = 7.6$ Hz, 1H, 4-H). $^{13}$C NMR ($CHCl_3$-$d_1$, 100.5 MHz): $\delta =$ 26.1 (C-8), 27.6 (C-9), 35.4 (C-7), 51.1 (C-11), 52.8 (C-13), 53.72 (C-10), 104.8 (C-5), 112.0 (C-3), 140.6 (C-4), 151.0 (C-6), 159.6 (C-2). For the $^1$H and $^{13}$C NMR spectra of the hydrochloride salt see Ref. [34]. HRMS. Found 268.0219. Calc. for $C_{11}H_{13}^{79}BrN_2O$: 268.0211. Found: 270.0191. Calc. for $C_{11}H_{13}^{81}BrN_2O$: 270.0190.

*6.2.8. (−)-5-Bromocytisin (11-bromo-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1.2-a][1.5]-diazocin-8-on) (**8b**)*

$[\alpha]_D^{20} = -10.9$ (c = 0.2, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) = 3442 (NH), 1650 (CO). UV($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 207 nm (3.94), 239 (3.76), 322 (3.66). $^1H$ NMR ($CHCl_3$-$d_1$, 400 MHz): $\delta = 1.62$ (s, broad, 1H, NH), 1.97 (m, 2H, 8-$H_\alpha$, 8-$H_\beta$), 2.32 (s, broad, 1H, 9-H), 2.93 (dd, $^2J = 12.0$ Hz, $^3J = 2.2$ Hz, 1H, 11-$H_\alpha$), 2.99 (d, $^2J = 12.2$ Hz, 1H, 13-$H_\alpha$), 3.08 (d, $^2J = 12.2$ Hz, 1H, 13-$H_\beta$), 3.19 (d, $^2J = 12.2$ Hz, 1H, 11-$H_\beta$), 3.34 (s, broad, 1H, 7-H), 3.91 (dd, $^2J = 15.6$ Hz, $^3J = 6.6$ Hz, 1H, 10-$H_\alpha$), 4.08 (d, $^2J = 15.6$ Hz, 1H, 10-$H_\beta$), 6.37 (d, $^3J = 9.7$ Hz, 1H, 3-H), 7.42 (d, $^3J = 9.5$ Hz, 1H, 4-H). For the $^1H$ NMR spectrum of the corresponding hydrochloride salt, see Ref. [34]. $^{13}C$ NMR ($CHCl_3$-$d_1$, 100.5 MHz): $\delta = 26.3$ (C-8), 27.3 (C-9), 34.7 (C-7), 50.2 (C-10), 50.8 (C-11), 52.6 (C-13), 98.5 (C-5), 117.6 (C-3), 142.4 (C-4), 148.0 (C-6), 162.4 (C-2). HRMS. Found: 268.0187. Calc. for $C_{11}H_{13}^{79}BrN_2O$: 268.0211. Found: 270.0177. Calc. for $C_{11}H_{13}^{81}BrN_2O$: 270.0190.

*6.2.9. (−)-3, 5-Diiodocytisine (9,11-diiodo-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido-[1.2-a][1.5] diazocin-8-one) hydrochloride (**9c**)*

To a stirred solution of **3** (190.2 mg, 1.00 mmol) in acetic acid (100%, 10 mL) was added dropwise a solution of iodine monochloride (1.38 g, 8.50 mmol) in acetic acid (100%, 10 mL) at room temperature within 8 min. After stirring for 4 h the solution was diluted with ice water (20 mL) and stirred vigorously for 16 h at room temperature. The crystalline precipitate was collected on a filter, washed twice with diethyl ether (5 mL), dried ($P_4O_{10}$) and recrystalised from ethanol (10 mL) to give compound **9c** as orange crystals (470 mg, 98%), m.p. 198–202°C (dec.). $[\alpha]_D^{20} = -11.2$ (c = 0.2, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) 3432 (NH), 1610 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 206 nm (4.05), 240 (4.03), 339 (3.73). $^1H$ NMR (DMSO-$d_6$, 400 MHz): $\delta = 1.97$ (m, 1H, 8-$H_\alpha$), 2.04 (m, 1H, 8-$H_\beta$), 2.59 (s, broad, 9-H), 3.23 (m, 2H, 13α-H, 11α-H), 3.33 (m, 2H, 13β-H, 11β-H), 3.41 (s, broad, 1H, 7-H), 3.87 (dd, $^2J = 15.2$ Hz, $^3J = 6.4$ Hz, lH, 10α-H), 3.98 (d, $^2J = 15.7$ Hz, 1H, 10β-H), 8.12 (s, broad, lH, NH), 8.34 (s, 1H, 4-H), 8.93 (s, broad, 1H, NH). $^{13}C$ NMR (DMSO-$d_6$, 100.5 MHz): $\delta = 22.9$ (C-8), 24.6 (C-9), 35.1 (C-7), 45.6* (C-10)[1], 47.4* (C-13), 50.5 (C-11), 70.9 (C-5), 91.0 (C-3), 146.7 (C-6), 154.4 (C-4), 159.2 (C-2). *Assignments not confirmed. MS. (70 eV) $m/z$ (%): 443 (8, $M^+ - Cl$), 442 (100). Anal. Found: C, 27.40; H, 2.71; N, 5.90. Calc. for $C_{11}H_{13}ClI_2N_2O$ (478.49): C, 27.61; H, 2.74; N, 5.85.

*6.2.10. Iodination of(−)-cytisine **3** with 1 equiv. mol of iodine monochloride to **6c**, **7c** and **8c***

To a stirred solution of **3** (570.7 mg, 3.00 mmol) in acetic acid (15 mL, 100%) was added dropwise a solution of iodine monochloride (487 mg, 3.00 mmol) in acetic acid (4.7 mL, 100%) at room temperature within 8 min. The mixture was stirred for 6 h and then water (20 mL) was added. After stirring for a further 16 h the solvent was removed in vacuo at 75–80°C (bath temperature) to give a yellow-brown, viscous oil (1198 mg). The crude material was dissolved in $CHCl_3$ (50 mL) and to the solution was added sodium carbonate (2 g). After 1 h, the suspension was filtered, and the solvent was removed under reduced pressure to yield a residue (5 mL), which was purified by silica gel column chromatography (18×3 cm, $CHCl_3/CH_3OH$ = 10:1), to give three fractions. Isolated yields were as follows: fraction 1 (**6c**), 12.2 mg (0.9%) as yellowish crystals, fraction 2, a mixture of the compounds **8c** and **7c** (483 mg, 51%) as a yellowish, viscous oil, fraction 3, (**7c**), as a yellow viscous oil (97 mg, 10%). Fraction 2 was purified again by silica gel column chromatography (20×2 cm, $CHCl_3/CH_3OH$ = 40:1) to give two fractions. Compounds **8c** (fraction 1′) and **7c** (fraction 2′) appeared to be a colourless viscous oil (177 mg, 19%) and a yellow viscous oil (236 mg, 25%), respectively.

*6.2.11. (−)-3-Iodocytisine (9-iodo-1,2,3,4,5, 6-hexahydro-1,5-methano-pyrido[1.2-a][1.5]-diazocin-8-one) fumarate (**10c**)*

A stirred solution of **7c** (75 mg, 0.24 mmol) in 2-propanol (2 mL) was heated to reflux and an excess of fumaric acid (41.8 mg, 0.35 mmol) dissolved in 2-propanol (3 mL) was added. The solution was kept at room temperature for 5 h, the precipitated solid was collected, washed three times with anhydrous diethyl ether (5 mL) and dried in vacuo over silica, yielding cream-coloured crystals (60.1 mg, 58%), m.p. 204–207°C. $[\alpha]_D^{20} = -71.2$ (c = 0.2, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) 3440 (NH), 2946, 1653 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 210 nm (4.30), 327 (4.03). $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta = 1.85$ (m, 2H, 8-$H_\alpha$, 8-$H_\beta$), 2.32 (s, broad, lH, 9-H), 2.86 (d, $^2J = 13.5$ Hz, 1H, 11-$H_\alpha$), 2.89 (d, $^2J = 14.5$ Hz, 13-$H_\alpha$), 2.94 (dd, $^2J = 11.8$ Hz, $^3J = 2.7$ Hz, 1H, 13-$H_\beta$), 3.00 (m, 2H, 11-$H_\beta$, 7-H), 3.78 (dd, $^2J = 15.3$ Hz, $^3J = 6.4$ Hz, lH, 10-$H_\alpha$), 3.89 (d, $^2J = 15.4$ Hz, 10-$H_\beta$), 5.95 (d, $^3J = 7.3$ Hz, lH, 5-H), 6.57 (s, 2H, vinyl-H), 7.97 (d, $^3J = 7.3$ Hz, 1H, 4-H). $^{13}C$ NMR (DMSO-$d_6$, 125.8 MHz): $\delta = 24.7$ (C-8), 26.6 (C-9), 33.6 (C-7), 50.5 (C-10), 51.1 (C-11), 52.1 (C-13), 86.7 (C-3),

105.7 (C-5), 134.0 (2 vinyl-C), 147.1 (C-4), 152.2 (C-6), 159.2 (C-2), 166.2 (2 carboxylate-C). Anal. Found: C, 41.52; H, 4.16; N, 6.48. Calc. for $C_{15}H_{17}IN_2O_5$ (432.84): C, 41.68; H, 3.96; N, 6.48%.

### *6.2.12. (−)-5-Iodocytisine (11-iodo-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1.2-a][1.5] -diazocin-8-one) fumarate **(11c)***

The compound **11a** was obtained by the same procedure as described for **l0c** from **8c** (22.7 mg, 0.07 mmol) and fumaric acid (12.7 mg, 0.10 mmol). Yield 12.4 mg (40%) colourless crystals, m.p. 149–152°C (dec.). $[\alpha]_D^{20} = -2.4$ (c = 0.2, $CH_3OH$); IR (KBr): $V$ ($cm^{-1}$) 3447 (NH), 2941 (CH), 1653 (CO). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon = 209$ nm (3.82), 237 (3.56), 324 (3.23). $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta = 1.88$ (s, broad, 2H, 8-$H_\alpha$, 8-$H_\beta$), 2.30 (s, broad, 1H, 9-H), 2.86 (d, $^2J = 11.9$ Hz, 2H, 11-$H_\alpha$, 13-$H_\alpha$), 2.99 (dd, $^2J = 12.1$ Hz, $^3J = 2.7$ Hz, 13-$H_\beta$), 3.02 (d, $^2J = 12.1$ Hz, lH, 11-$H_\beta$), 3.15 (s, broad, 1H, 7-H), 3.75 (dd, $^2J = 15.5$ Hz, $^3J = 6.5$ Hz, 1H, 10-$H_\alpha$), 3.82 (d, $^2J = 15.3$ Hz, lH, 10-$H_\beta$), 6.08 (d, $^3J = 9.4$ Hz, 1H, 3-H), 6.58 (s, 2H, vinyl-H), 7.64 (d, $^3J = 9.6$ Hz, 1H, 4-H). $^{13}C$ NMR (DMSO-$d_6$, 125.8 MHz): $\delta = 25.7$ (C-8), 26.6 (C-9), 38.1 (C-7), 49.1 (C-10), 50.4 (C-11)[1], 51.2 (C-13)[1], 69.2 (C-5), 117.8 (C-3), 134.1(2 vinyl-C), 147.3 (C-4), 150.1 (C-6), 161.7 (C-2), 166.2 (2 carboxylate-C). Anal. Found: C, 41.42; H, 4.07; N, 6.26. Calc. for $C_{15}H_{17}IN_2O_5$ (432.84): C, 41.68; H, 3.96; N, 6.48%.

### *6.2.13. (−)-Thiocytisine (1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1.2-a][1.5]-diazocin-8-thione) **(12)***

A mixture of (−)-cytisine (**3**) (193 mg, 1.00 mmol) and Lawesson's reagent (0.50 mmol) was taken in a glass tube and mixed thoroughly with a spatula. The glass tube was placed in an alumina bath inside the microwave oven (800 W) and irradiated for 3 min. The coloured crude material was dissolved in $CHCl_3$ (50 mL), the resulting solution filtered, and the solvent removed to give a residue (3 mL); this was adsorbed on alumina (3 g) and purified by alumina column chromatography (20 cm×3 cm, $CHCl_3/CH_3OH = 40:1$) affording the pure compound **12**; yield 78.5 mg (37%) pale yellow crystals, m.p. 163–165°C, $R_f = 0.45$ (silica gel, $CHCl_3$–$CH_3OH = 5:1$). $[\alpha]_D^{20} = -244.7$ ($c = 0.2$, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) = 3426 (NH), 1206 (C = S). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 209 nm (3.88), 286 (3.92), 357 (3.85). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta = 1.54$ (s, broad, 1H, NH), 1.98 (m, 2H, 8-$H_\alpha$, 8-$H_\beta$), 2.47 (s, broad, 1H, 9-H), 2.99 (dd, $^2J = 9.7$ Hz, $^3J = 1.1$ Hz, 11-$H_\alpha$), 3.03 (s, broad, 1H, 7-H), 3.05 (dd, $^2J = 7.2$ Hz, $^3J = 1.8$ Hz, 1H, 13-$H_\alpha$), 3.10 (dd, $^2J = 7.1$ Hz, $^3J = 1.6$ Hz, 1H, 13-$H_\beta$), 3.12 (d, $^2J = 11.9$ Hz, 1H, 11-$H_\beta$), 4.26 (dd, $^2J = 16.3$ Hz, $^3J = 6.6$ Hz, 1H, 10-$H_\alpha$), 4.63 (d, $^2J = 16.1$ Hz, 1H, 10-$H_\beta$), 6.52 (dd, $^3J = 7.1$ Hz, $^4J = 1.6$ Hz, 1H, 5-H), 7.17 (dd, $^3J = 8.5$ Hz, $^3J = 7.1$ Hz, 1H, 4-H), 7.69 (dd, $^3J = 8.6$ Hz, $^4J = 1.5$ Hz, 1H, 3-H). $^{13}C$ NMR ($CHCl_3$-$d_1$, 100.5 MHz): $\delta = 25.8$ (C-8), 28.8 (C-9), 36.6 (C-7), 52.8 (C-13), 53.8 (C-11), 58.1 (C-10), 113.6 (C-5), 133.3 (C-4), 133.5 (C-3), 154.3 (C-6), 179.8 (C-2). MS (70 eV) $m/z$ (%): 208 (5, $M^+ + 2$), 207 (14, $M^+ + 1$), 206 (100, $M^+$). HRMS. Found: 206.0879. Calc. for $C_{11}H_{14}N_2S$: 206.0877.

### *6.2.14. (−)-12-Acetyl-thiocytisine (3-acetyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido-[1.2-a][1.5]diazocin-8-thione) **(13)***

To a stirred solution of **12** (50.9 mg, 0.25 mmol) in $CH_2Cl_2$ (8 mL) was added $Na_2CO_3$ (212 mg, 2.00 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol) and dropwise a solution of acetylchloride (0.02 mL, 0.28 mmol) in $CH_2Cl_2$ (2 mL). After stirring for 16 h at room temperature the suspension was filtered, the residue washed three times with $CH_2Cl_2$ (10 mL), and the resulting solution was concentrated in vacuo to yield 72.5 mg of a semicrystalline solid. The product was purified by silica gel chromatography (20.5 cm×3 cm, $CHCl_3/CH_3OH = 10:1$) to give compound **13** as yellow crystals (41 mg, 67%), m.p. 153–155°C. $[\alpha]_D^{20} = -522.9$ ($c = 0.1$, $CH_3OH$); IR (KBr): $\nu$ ($cm^{-1}$) = 2915 (CH), 1640 (CO), 1611, 1544, 1206 (C = S). UV ($CH_3OH$): $\lambda_{max}$ (log $\varepsilon$) = 209 nm (4.04), 288 (3.94), 359 (3.84). $^1H$ NMR ($CHCl_3$-$d_1$, 400 MHz, two rotamers, ratio 2:1): $\delta = 1.74$ (s, 3H, COC$H_3$), 2.01 (s, 3H, COC$H_3$), 2.07 (s, broad, 4H, 8-$H_\alpha$, 8′-$H_\beta$), 2.67 (s, broad, 2H, 9-H, 9′-H), 2.83 (d, $^2J = 13.2$ Hz, 1H, 11-1H, 11-$H_\alpha$), 2.89 (dd, $^2J = 13.4$ Hz, $^3J = 2.0$ Hz, 1H, 13-$H_\alpha$), 3.24 (s, broad, 2H, 7-H, 7′-H), 3.42 (d, $^2J = 13.4$ Hz, 1H, 13-$H_\beta$), 3.47 (dd, $^2J = 13.2$ Hz, $^3J = 2.0$ Hz, 1H, 11-$H_\beta$), 3.87 (d, $^2J = 13.0$ Hz, 1H, 11-$H_\alpha$), 3.98 (d, $^2J = 13.2$ Hz, 1H, 13-$H_\alpha$), 4.23 (dd, $^2J = 16.5$ Hz, $^3J = 6.6$ Hz, 2H, 10-$H_\alpha$, 10′-$H_\alpha$), 4.56 (d, $^2J = 16.3$ Hz, 1H, 10-$H_\beta$), 4.68 (d, $^2J = 13.2$ Hz, 1H, 13′-$H_\beta$), 4.74 (d, $^2J = 16.5$ Hz, 1H, 10′-$H_\beta$), 4.78 (d, 1H, 11′-$H_\beta$), 6.61 (m, $^3J = 7.1$ Hz, $^4J = 1.5$ Hz, 2H, 5-H, 5′-H), 7.16 (2d, $^3J = 8.6$ Hz, $^3J = 7.1$ Hz, 2H, 4-H, 4′-H), 7.66 (2d, $^3J = 8.6$ Hz, $^4J = 1.5$ Hz, 2H, 3-H, 3′-H). $^{13}C$ NMR ($CHCl_3$-$d_1$, 100.5 MHz, two rotamers, ratio 2:1): $\delta$ 20.8 (CO$CH_3$), 21.2 (CO$CH_3$), 25.4 (C-8, C-8′), 28.2 (C-9′), 28.5 (C-9), 35.4 (C-7), 35.9 (C-7′), 47.3 (C-11′), 48.3 (C-13), 52.3 (C-11), 53.4 (C-13′), 56.9 (C-10), 57.0 (C-10′), 113.2 (C-5′), 114.4 (C-5),

132.8 (C-4′), 133.5 (C-4), 134.0 (C-3), 134.4 (C-3′), 151.4 (C-6′), 151.5 (C-6), 169.5 ($COCH_3'$), 169.7 ($COCH_3$), 179.9 (C-2), 180.8 (C-2′). MS. (70 eV) $m/z$ (%): 250 (6, $M^+ + 2$), 249 (16, $M^+ + 1$), 248 (100, $M^+$); HRMS. Found: 248.0996. Calc. for $C_{13}H_{16}N_2SO$: 248.0983.

### 6.3. Crystal-structure determination

#### 6.3.1. Preparation of single crystals for X-ray analysis

Compound **12** (10 mg) was dissolved in $CHCl_3$ (0.5 mL) and filtered through a folded filter paper in such a way that the filtrate directly went into a test tube (bore ca. 1 cm). Then cyclo-hexane (3 mL), as a suitable precipitant, was layered carefully down the side of the tube on to the solution. The tube was then corked and left to stand undisturbed for 12 h, furnishing yellow crystals suitable for X-ray crystallographic analysis.

#### 6.3.2. Crystal-structure determination of **12**

A yellow prism crystal (ca. $0.50 \times 0.50 \times 0.30$ mm), obtained by recrystallisation from $CHCl_3$/cyclohexane by the liquid diffusion method, was mounted on a glass fiber and investigated on a Rigaku AFC5R diffractometer with graphite monochromated Cu Kα radiation and a rotating anode generator (Rigaku). Empirical formula $C_{11}H_{14}N_2S$, molecular mass 206.30 a.u. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $77.63 < 2\Theta < 79.85°$ corresponded to a primitive orthorhombic cell with dimensions $a = 11.271(2)$, $b = 12.622(3)$, $c = 7.147(3)$ and $V = 1016.8(5)$ Å$^3$. For $Z = 4$ and F.W. = 206.30, the calculated density is 1.35 g cm$^{-3}$. The systematic absences of: $h00$: $h \neq 2n$, $0k0$: $k \neq 2n$, $001$: $l \neq 2n$ uniquely determine the space group to be: $P2_12_12$ ($\#19$). The data were collected at a temperature of $23 \pm 1$°C using the $\omega$–$2\Theta$ scan technique to a maximum $2\Theta$ value of 120.5°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.26° with a take-off angle of 6.0°. Scans of $(1.37 + 0.30 \tan \Theta)$° were made at a speed of 32.0° min$^{-1}$ (in omega). The weak reflections ($I < 10.0\sigma(I)$) were rescanned (maximum of 4 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 1.0 mm, the crystal to detector distance was 400 mm, and the detector aperture was 9.0 mm × 13.0 mm (horizontal × vertical). A total of 925 reflections was collected. The intensities of three representative reflections were measured after every 150 reflections. No decay correction was applied. The linear absorption coefficient, $\mu$, for Cu-Kα radiation is 24.8 cm$^{-1}$. An empirical absorption correction based on azimuthal scans of several reflections was applied, which resulted in transmission factors ranging from 0.84 to 1.00. The data were corrected for Lorentz and polarisation effects.

The structure was solved by direct methods [55] and expanded using Fourier techniques [56]. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included but not refined. The final cycle of full matrix least-squares refinement[2] was based on 872 observed reflections ($I > 3.00\sigma(I)$) and 127 variable parameters and converged (largest parameter shift was 0.03 times its e.d.s.) with unweighted and weighted agreement factors of $R = \Sigma \| F_o | - | F_c \| / \Sigma | F_o | = 0.037$; and $R_w = \sqrt{(\Sigma_\varpi (|F_o| - |F_c|)^2 / \Sigma_\varpi F_o^2)} = 0.039$. The standard deviation of an observation of unit weight was 3.39. The weighting figure was based on counting statistics and included a factor ($p = 0.04$) to downweight the intense reflections. Plots of $\Sigma\ \varpi(|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, $\sin\theta/\lambda$ and the various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.17 and 0.21 e$^-$Å$^{-3}$, respectively.

The neutral atom scattering factors were taken from Cromer and Waber [57]. Anomalous dispersion effects were included in Fcalc [58]; The values for $\Delta f'$ and $\Delta f'$ were those of Creagh and McAuley [59]. The values for the mass attenuation coefficients are those of Creagh and Hubbell [60]. All calculations were performed using the teXsan [61] crystallographic software package of Molecular Structure Corporation.

### 6.4. In vitro receptor binding

#### 6.4.1. Materials

(±)-[$^3$H]Epibatidine (33 Ci mmol$^{-1}$) was obtained from NEN Life Science Products (Cologne, Germany). [$^3$H]MLA (20 Ci mmol$^{-1}$) was purchased from Tocris/

[2] Least-squares: functional minimized $(\Sigma\varpi(|F_o| - |F_c|)^2$ where $w = 1{:}\sigma_c^2(F_o) = [\sigma_c^2(F_o) + p2{:}4F_o^2]^{-1}$, $\sigma_c(do)$ = e.s.d. based on counting statistics; $p$ = p-factor. Standard deviation of an observation of unit weight: $\sqrt{(\Sigma\varpi(|F_o| - |F_c|)^2/(No - Nv)}$ where: No = number of observations, $Nv$ = number of variables.

Biotrend (Cologne, Germany). All other chemicals used were obtained from Sigma-Aldrich (Deisenhofen, Germany). Frozen Sprague–Dawley rat brains were purchased from Pel-Freez Biologicals (Rogers, AR).

*6.4.2. Membrane preparation*

Frozen rat brains were thawed at 22°C for 30–60 min before membrane preparation. A crude membrane fraction (P2) was isolated as previously described [15, 31, 35] and stored in aliquots at −80°C for at least 16 h but no more than 4 weeks before use. The pellets of the crude membrane fraction were washed just once on the day of assay. The pellets were homogenised in 30 volumes of a HEPES–salt solution (HSS) containing HEPES (15 mM), NaCl (120 mM), KCl (5.4 mM), $MgCl_2$ (0.8 mM), and $CaCl_2$ (1.8 mM). After centrifugation at 35,000×*g* for 10 min, the resultant pellets were resuspended in a fresh HSS and used for binding assay.

*6.4.3. $(\alpha 4)_2(\beta 2)_3$ Binding assay*

Binding assay was performed following a published procedure [15]. Briefly, assays were carried out in HSS at 22°C and performed in duplicate. Nonspecific binding was determined in the presence of 300 μM (−)-nicotine. Each assay sample of a total volume of 0.5 mL contained 60 μg of membrane protein, 0.5 nM (±)-[$^3$H]epibatidine, and 0.2 mL of a test compound. The samples were incubated for 90 mm, and the incubation were terminated by vacuum filtration through Whatman GFIB glass fiber filters, presoaked in 1% poly(ethylenimine) using a Brandel 48-channel cell harvester. The radioactivity was measured using a liquid scintillation counter (Tri-Carb 2100 TR, Packard, Dreieich, Germany).

*6.4.4. α7* Binding assay*

The procedure used in the radioligand binding assay was reported in detail by Davies et al. [36] with minor modifications. Nonspecific binding was measured in the presence of 1 μM MLA (methyllycaconitine). Each assay sample contained 50 μL of the test compound, 100 μL [$^3$H]MLA to achieve a final concentration of 2 nM, and 100 μL resuspended membranes. The samples were incubated for 180 min at 22°C and the incubation was terminated by rapid filtration under vacuum through Whatman GFIB filters presoaked in 1% poly(ethylenimine). The radioactivity bound to the filters was measured by a liquid scintillation counter.

*6.4.5. Data analysis*

Competition binding data were analysed using nonlinear regression methods. $K_i$ values were calculated by the Cheng–Prusoff [62] equation based on the measured $IC_{50}$ values and $K_d = 10$ pM for binding of (±)-[$^3$H]epibatidine and $K_d = 1$ nM for [$^3$H]MLA. The $K_d$ values were obtained from five independent experiments preformed on the same membrane preparations that were used for the competition assays.

## 7. Supplementary material

Crystallographic data (excluding structure factors) for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-144569. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.ca-m.ac.uk).

## Acknowledgements

Financial support from Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged. We thank Bayer, A.G., Merck, A.G., and Degussa, A.G. for supplying us with valuable chemicals.

## References

[1] Lloyd G.K., Williams M.J., Pharmacol. Exp. Ther. 292 (2000) 461–467 (and the literature cited therein).

[2] Schmitt J.D., Bencherif M. (Eds.), Annual Reports in Medicinal Chemistry, vol. 35, Academic Press, New York, 2000, pp. 41–52.

[3] Gispen W.H., Bruinwels J., Nijkamp P., Versteeg D.H.G. (Eds.), Eur. J. Pharmacol. 393 (1–3) (2000) 1–340.

[4] Holladay M.W., Dart M.J., Lynch J.K., J. Med. Chem. 40 (1997) 4169–4194.

[5] Prendergast M.A., Jackson W.J., Terry A.V. Jr., Decker M.W., Arneric S.P., Buccafusco J.J., Psychopharmacology 136 (1998) 50–58.

[6] Ghelardini C., Galeotti N., Barlocco D., Bartolini A., Drug Dev. Res. 40 (1997) 251–258.

[7] Menzaghi F., McClure D.A., Lloyd G.K., in: Arneric S.P., Brioni J.D. (Eds.), Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities, Wiley–Liss, New York, 1998, pp. 379–394.

[8] Levin E.D., Bettergowda C., Blosser J., Gordon J., Behav. Pharmacol. 10 (1999) 675–680.

[9] McDonald I.A., Vernier J.M., Cosford N., Corey-Naeve J., Curr. Pharmacol. Des. 2 (1996) 357–366.

[10] Badio B., Garaffo H.M., Spande T.F., Daly J.W., Med. Chem. Res. 4 (1994) 440–448.

[11] Bai D., Xu R., Zhu X., Drugs Future 22 (1997) 1210–1220.

[12] Wright E., Gallagher T., Sharples C.G.V., Wonnacott S., Bioorg. Med. Chem. Lett. 7 (1997) 2867–2870.

[13] Badio B., Garaffo H.M., Plummer C.V., Padgett W.L., Daly J.W., Eur. J. Pharmacol. 321 (1997) 189–194.

[14] Bannon A.W., Decker M.W., Holladay M.W., Curzon P., Donnelly-Roberts D., Puttfarcken P.S., Bitner R.S., Diaz A., Dickenson A.H., Williams M., Arneric S.P., Science 279 (1998) 77–81.

[15] Koren A.O., Horti A.G., Mukhin A.G., Gündisch D., Kimes A.S., Dannals R.F., London E.D., J. Med. Chem. 41 (1998) 3690–3698.

[16] Williams M., Sullivan J.P., Arneric S.P., Drug News Perspect. 7 (1994) 205.

[17] Perry E.K., Morris C.M., Court J.A., Cheng A., Fairbairn A.F., McKeith I.G., Irving D., Brown A., Perry R.H., Neuroscience 64 (1995) 385–395.

[18] Lin N.-H., Meyer M.D., Exp. Opin. Ther. Patents 8 (1998) 991–1015.

[19] Leonard N.I., in: Manske R.H.F., Holmes H.L. (Eds.), The Alkaloids, vol. 3, Academic Press, New York, 1953, pp. 119–199.

[20] Ohmiya S., Saito K., Murakoshi I., in: Cordell G.A. (Ed.), The Alkaloids, vol. 47, Academic Press, New York, 1995, pp. 1–114.

[21] Bojadschiewa M., Issaev I., Totev I., Dimov C., Pharmazie 26 (1971) 643.

[22] Anderson D.J., Arneric S.P., Eur. J. Pharmacol. 253 (1994) 261–267.

[23] Glennon R.A., Dukat M., Med. Chem. Res. (1996) 465–486.

[24] Hall M., Zerbe L., Leonard S., Freedman R., Brain Res. 600 (1993) 127–133.

[25] Schmeller T., Sauerwein M., Sporer F., Wink M., Müller W.E., J. Nat. Prod. 57 (1994) 1316–1319.

[26] Sloan J.W., Martin W.R., Bostwick M., Hook R., Wala E., Pharmacol. Biochem. Behav. 30 (1988) 255–267.

[27] Boido Canu C., Sparatore F., Farmaco 54 (1999) 438–451.

[28] Che D., Siegl J., Seitz G., Tetrahedron: Asymmetry 10 (1999) 573–585.

[29] Che D., Wegge T., Stubbs M.T., Seitz G., Meier H., Methfessel C., J. Med. Chem. 44 (2001) 47–57.

[30] Naumann K., J. Prakt. Chem. 341 (1999) 417–435.

[31] Mukhin A.G., Gündisch D., Horti A.G., Koren A.O., Tamagnan G., Kimes A.S., Chambers J., Vaupel D.B., King S., Picciotto M.R., Innis R., London E.D., Mol. Pharm. 57 (2000) 642–649.

[32] Patani G.A., LaVoie E.J., Chem. Rev. 96 (1996) 3147–3176.

[33] Marriere E., Rouden J., Tadino V., Lasne M.C., Org. Lett. 2 (2000) 1121–1124.

[34] (a) O'Neill B.T., PCT Int. Appl. WO 98 18,798, 1998. (b) O'Neill B.T., Chem. Abstr. 119 (1998) 4774k.

[35] Gündisch D., London E.D., Terry P., Hill G.R., Mukhin A.G., Neuroreport 10 (1999) 1631–1636.

[36] Davies A.R.L., Hardick D.J., Blagbrough I.S., Potter B.V.L., Wolstenholme A.J., Wonnacott S., Neuropharmacology 38 (1999) 679–690.

[37] Okuda S., Tsuda K., Kataoka H., Chem. Ind. (London) (1961) 1751.

[38] (a) van Tamelen E.E., Baran J.S., J. Am. Chem. Soc. 77 (1955) 4944–4955. (b) van Tamelen E.E., Baran J.S., J. Am. Chem. Soc. 78 (1956) 2913–2914. (c) van Tamelen E.E., Baran J.S., J. Am. Chem. Soc. 60 (1958) 4659–4670.

[39] Bohlmann F., Englisch A., Ottawa N., Sander H., Weise W., Angew. Chem. 67 (1955) 708.

[40] Govindachari T.S., Rajadurai S., Subramanian M., Thyagarajan B.S., J. Chem. Soc. (1957) 3839–3844.

[41] O'Neill B.T., Yohannes D., Bundesmann M.W., Arnold E.P., Org. Lett. 2 (2000) 4201–4204.

[42] Coe J.W., Org. Lett. 2 (2000) 4205–4208.

[43] Mascagni P., Christodoulou M., Gibbons W.A., Asres K., Phillipson J.D., Niccolai N., Mangani St., J. Chem. Soc. Perkin Trans. II (1987) 1159–1165.

[44] Orjales A., Ribas L., Varela A., An. Quim. 68 (1972) 1419–1432.

[45] Hartke K., Gerber H.D., J. Prakt. Chem. 338 (1996) 763–765.

[46] Varma R.S., Kumar D., Org. Lett. 1 (1999) 697–700.

[47] Spinner E., J. Chem. Soc. (1960) 1237–1242.

[48] Seitz G., Mann K., Schmiedel R., Matusch R., Chem. Ber. 112 (1979) 990–999.

[49] Mayer R., Schinke E., Rosmus P., Scheithauer S., J. Prakt. Chem. 312 (1970) 767–775.

[50] Borowiak T., Dutkiewicz G., Wysocka W., Kolanos R., Z. Naturforsch. B 54 (1999) 1307–1312.

[51] Allen F.H., Bird C.M., Rowland R.S., Raithby P.R., Acta Crystallogr., Sect. B 53 (1997) 680–695.

[52] Cox C., Lectka T., J. Org. Chem. 63 (1998) 2426–2427.

[53] Sheridan R.P., Nilakantan R., Dixon J.S., Venkataraghavan R., J. Med. Chem. 29 (1986) 899–906.

[54] Luputiu G., Moll F., Arch. Pharm. 304 (1971) 151–158.

[55] Altomare A., Cascarano M., Giacovazzo C., Guagliardi A., Completion and refinement of crystal structures with SIR92, J. Appl. Crystallogr. 26 (1993) 343–350.

[56] DIRDIF-94: P.T. Beurskens, G. Admiraal, G. Beurskens, W.P. Bosman, R. de Gelder, R. Israel, J.M.M. Smits, The DIRDIF-94 program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1994.

[57] Cromer D.T., Waber J.T., International Tables for X-ray Crystallography, vol. IV, The Kynoch Press, Birmingham, 1974 (Table 2.2.A).

[58] Ibers J.A., Hamilton W.C., Acta Crystallogr. 17 (1964) 781–782.

[59] Creagh D.C., McAuley W.J., in: Wilson A.J.C. (Ed.), International Tables for Crystallography, vol. C, Kluwer Academic, Boston, 1992, pp. 219–222 (Table 4.2.6.8).

[60] Creagh D.C., Hubbell J.H., in: Wilson A.J.C. (Ed.), International Tables for Crystallography, vol. C, Kluwer Academic, Boston, 1992, pp. 200–206 (Table 4.2.4.3).

[61] teXsan: Crystal Structure Analysis Package, Molecular Structure Corporation (1985 & 1992).

[62] Cheng Y.-C., Prusoff W.H., Biochem. Pharmacol. 22 (1973) 3099.