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REVIEW

Cytisine: a natural product lead for the development of drugs acting at nicotinic acetylcholine receptors

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This review covers classical and modern structural modifications of the alkaloid, the more recent (since 2007) syntheses of cytisine and analogues, and the pharmacology of these compounds, with emphasis on their interactions with nicotinic receptors. 89 references are cited.

1 Introduction

(−)-Cytisine ((1*R*,5*S*)-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,2]diazocin-8-one, Fig. 1) may be regarded as the prototype of the tricyclic quinolizidine alkaloids of the legume family. It is widespread in the Faboideae, often accumulates in the seeds, and is obtained on a commercial scale from *Laburnum anagyroides* Medik, *Sophora alopecuroides* L., *Thermopsis alterniflora* Regel & Schmalh., *Thermopsis lanceolata* R. Br., and *Caragana sinica* (Buc'hoz) Rehder. Although it was first isolated in the mid-19th century,[1] its structure (without stereochemistry) was only determined in 1932,[2] and its absolute configuration was demonstrated almost thirty years later.[3] X-Ray crystal structures are available for cytisine and *N*-methylcytisine, and the structures of cytisine in solution and in the crystal phase are congruent.[4–6] Although the cytisine skeleton has traditionally been numbered starting at the pyridone nitrogen atom, some authors prefer to use the systematic numbering advocated by the IUPAC.

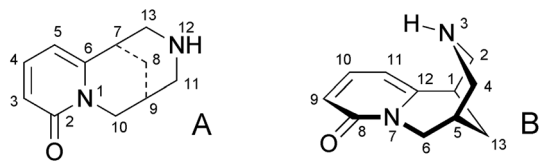

Fig. 1 Structure and numbering of cytisine. A) projection structure with traditional numbering; B) three-dimensional structure with IUPAC numbering.

Although some other activities have been recorded, cytisine is best known – and has been most studied – with regard to its interaction with nicotinic acetylcholine receptors, which are involved in a large array of pathologies. The main current interest in this alkaloid seems to arise from its use as an aid to quit tobacco smoking, for which it is still marketed, at least in Poland and Russia,[7] but its activity is far from optimal and its molecular scaffold is therefore an inspiration for the development of more effective drugs.

2 Structural modification of cytisine

The earliest known modifications of (−)-cytisine date back to the late 19th century. In connection with its structure assignment, it was proposed to be a secondary amine after conversion into its nitroso, acetyl, *N*-methyl- and *N*-ethyl derivatives.[8,9] Cytisine was also shown to give a "nitronitrosocytisine" and dibromo and dichloro derivatives, and later a monobromocytisine, dibromo-*N*-methylcytisine, and its methiodide.[10,11] "Nitronitrosocytisine" was found to be accompanied by an isomer, both forms ("α" and "β") were hydrolysed giving the respective nitrocytisines, and reduction of the former led to an aminocytisine, which was diacetylated; treatment with formaldehyde gave a "methylenedicytisine". Moreover, α-nitronitrosocytisine could be brominated to produce an α-bromonitronitrosocytisine, which, like its precursor, underwent hydrolysis giving α-bromonitrocytisine; treatment of dibromocytisine with nitric acid only gave dibromonitrosocytisine, confirming that nitro and bromo

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substitutions only occurred at two positions on the cytisine molecule. Finally, electrolytic reduction of cytisine afforded a compound described as tetrahydrodeoxycytisine.[12,13] In the course of the structural studies that led finally to the accepted cytisine structure, a number of products of Hofmann elimination, ozonolysis and reduction of the alkaloid were prepared in the early 1930's.[2]

Apart from the *N*-nitroso and *N*-Boc groups used in recent years for protection of the secondary amine function, an *N*-phenylthiocarbamyl derivative was prepared as long ago as 1900,[14] and several phenyl-substituted analogues of this compound were obtained 70 years later.[15] The preparation of *N*-2-hydroxyethylcytisine, its benzoate and cinnamate, and some aromatic esters of *N*-3-hydroxypropylcytisine was reported in 1936.[16] However, most of the currently known *N*-substituted cytisine derivatives were prepared about a decade ago by Sparatore's group.[17–19]

Edwin G. Pérez

Edwin G. Pérez was born in Corozal, Colombia, and received his MSc in natural products chemistry in 2003 under the supervision of Jairo Sáez, from the University of Antioquia (Medellín, Colombia). He was then awarded a DAAD (German Academic Exchange Service) scholarship to undertake PhD studies at the University of Chile with Bruce Cassels, graduating in 2008. His postdoctoral experience includes research visits to the laboratory of Kilian Muñiz at the ICIQ in Tarragona (Spain), and he is currently an assistant professor at the Pontificial Catholic University of Chile. His research interests include the synthesis of nicotinic ligands.

Carolina Méndez-Gálvez

Carolina Méndez was born and educated in Santiago, Chile, where she graduated with the degree of "Licenciado" in chemistry from the University of Santiago in 2008. She immediately joined her university's chemistry PhD programme and is now completing her thesis research under the joint supervision of Edwin G. Pérez and Bruce Cassels in the latter's laboratory. As part of her doctoral studies she spent a short period working with Julio Seijas, at the University of Santiago de Compostela (Spain). Her current project involves the synthesis of potential nicotinic ligands and their structure–activity relationships.

The structures of dibromo- and dichlorocytisine were only demonstrated in 1971 and 1972, respectively.[20,21] Freund and Horkheimer's isomeric nitrocytisines have been prepared more recently *via* their *N*-nitroso derivatives and shown to be 3- and 5-nitrocytisines,[22] and 3-aminocytisine and *N*-acetyl-3,5-dinitrocytisine have also been obtained.[19] The X-ray crystal structure of 3-nitrocytisine has been published.[23] Monohalogenation at C3 and C5 of either *N*-protected or unprotected cytisine has also only been reported recently, including the iodo compounds.[22,24,25] The less easily accessible fluorocytisines were obtained in low yields even more recently by direct fluorination.[26] Nitration of 3- and 5-bromocytisines afforded 3-bromo-5-nitro- and 5-bromo-3-nitrocytisines, respectively.[26] A recent preparation of di-N^{12}cytisinylmethane (presumably Freund and Friedmann's "methylenedicytisine"),[12] has been reported with no details.[17] Some of these "classical" derivatives have served as starting points for "modern" reactions. Thus, 3-methyl, 3-vinyl-, 3-(4-fluorophenyl)-, 3-(4-butylphenyl)-, 3-(2-nitropyridyl)-, 3-(2-fluoropyridyl)- and 3-(5-methyl-2-thienyl)cytisine were all synthesised starting from *N*-protected 3-bromocytisine.[22,28,29]

The carbonyl oxygen of cytisine was replaced by sulfur, using Lawesson's reagent, affording thiocytisine.[24] An unusual lithium diisopropylamide-induced acyl migration from the secondary amine nitrogen and alkylation of *N*-benzylcytisine afforded several different C10-substituted derivatives.[30,31]

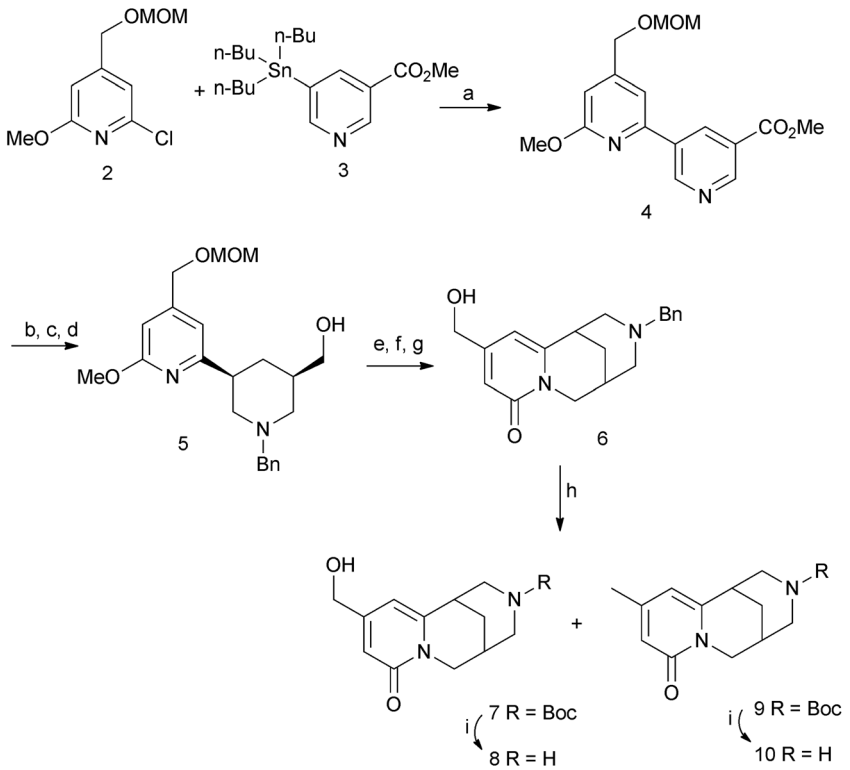
3 Total syntheses of cytisine and analogues

The synthesis of cytisine itself has been reviewed recently.[32] A single new synthesis has been published since then, which also provided access to novel core analogues, such as benzo[3,4] cytisine and 4-azacytisine (Schemes 10–12).[33] A synthesis of 2,3,4,5-tetrahydrocytisine appeared recently,[34] and ring-contracted analogues have also been synthesised in addition to the *carba*- analogues reviewed by Coe *et al.*[35–37]

As seen in the previous section, *N*-substitution and electrophilic substitution at C3 and C5 are quite straightforward. Access to other positions, however, requires "total" synthesis.

Bruce K. Cassels

Bruce Cassels was born in Buenos Aires, Argentina. He received his PhD from the University of Buenos Aires in 1966 and then took up a position at the State Technical University in Santiago, Chile. He was a postdoc at Bonn University (Germany), and spent periods working with Maurice Shamma at Penn State. In 1982–83 he taught at SUNY-Stony Brook and subsequently worked at the Universities of Paris-XI (with André Cavé) and Munich (with Meinhart Zenk). In 1988 he accepted a University of Chile professorship and started a program in medicinal chemistry, concentrating on CNS-active compounds and bioactive natural product derivatives.



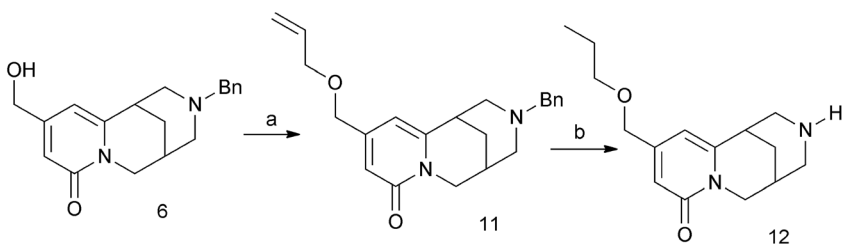
Scheme 1 Reagents and conditions: (a) $Pd(PPh_3)_4$, DMF, 130 °C, 15 h, 79%; (b) $LiAlH_4$, THF, −20 °C, 3.5 h, 59%; (c) BnBr, CH_3CN, reflux, 2 h; (d) H_2 (1 atm), PtO_2, Et_3N, MeOH, rt, 15 h, *cis* : *trans* = 5 : 1; *cis*, 67%; (e) MsCl, Et_3N, DCM, 0 °C, 30 min, 84%; (f) toluene, reflux, 3 h, 83%; (g) TFA, rt, 3 h, 91%; (h) H_2 (1 atm), 20% Pd–C (0.1 equiv), $(Boc)_2O$, MeOH, reflux, 30 min, 92%; (i) TFA, CH_2Cl_2, rt, 1 h, 87–93%.

Several approaches to this problem have been published in the last few years. A synthesis of 4-methyl- and 4-hydroxymethylcytisine[29] was followed closely by substitution products of the latter.[38] Their approach was based on the synthesis of cytisine and 3-methoxycytisine by O'Neill, inspired by van Tamelen's pioneering approach,[39,40] and began with a Pd-catalyzed Stille coupling of 2-chloro-6-methoxy-4-methoxymethoxymethylpyridine (**2**) with 3-methoxycarbonyl-5-tributylstannylpyridine (**3**) (Scheme 1) to afford a methoxybipyridinyl ester (**4**), which was reduced (both the ester and the pyridine ring) giving 3,5-*cis*-piperidine **5**. This was cyclized using the O'Neill strategy with some modifications, with final deprotection of the *O*-MOM group to afford *N*-benzyl-10-(hydroxymethyl)cytisine (**6**). Palladium-catalyzed hydrogenolysis in the presence of $(Boc)_2O$ afforded a mixture of the 4-hydroxymethyl derivative (**7**) and the over-reduced product (**9**). Finally, *N*-Boc deprotection of **7** with TFA gave the 4-substituted racemic cytisine derivatives **8** and **10**. Optimised conditions (20% $Pd(OH)_2$–C, (0.1 eq), H_2 (1 atm), $(Boc)_2O$, MeOH, 5 min, reflux) avoided over-reduction whilst still giving *N*-debenzylation which proceeded smoothly to afford *N*-Boc-10-hydroxymethylcytisine (**7**) in 97% yield.

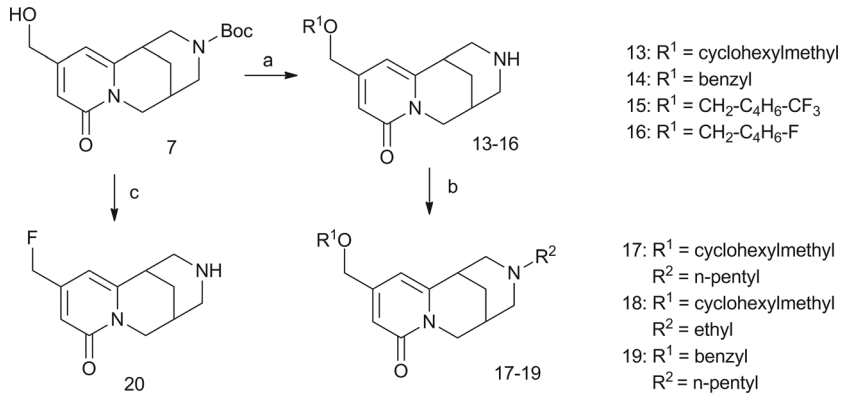
10-Propyloxymethylcytisine (**12**) was synthesised using *N*-benzyl-10-(hydroxymethyl) cytisine (**6**) as the precursor (Scheme 2). Thus, the reaction of allyl bromide with **6** gave the *O*-allyl ether derivative (**11**), which upon hydrogenation followed by *N*-Boc-deprotection of the intermediate afforded **12** in good yield.

N-Boc-10-(hydroxymethyl)cytisine (**7**) was used to synthesise the cyclohexylmethyl and benzyl ether derivatives (**13–16**) using NaH as base and the corresponding alkyl halides (Scheme 3). The Boc group was removed and subsequent treatment with 1-bromopentane or ethyl bromide gave compounds **17–19**.

The conversion of the hydroxymethyl group to fluoromethyl (**20**), was achieved using diethylaminosulphurtrifluoride (DAST).



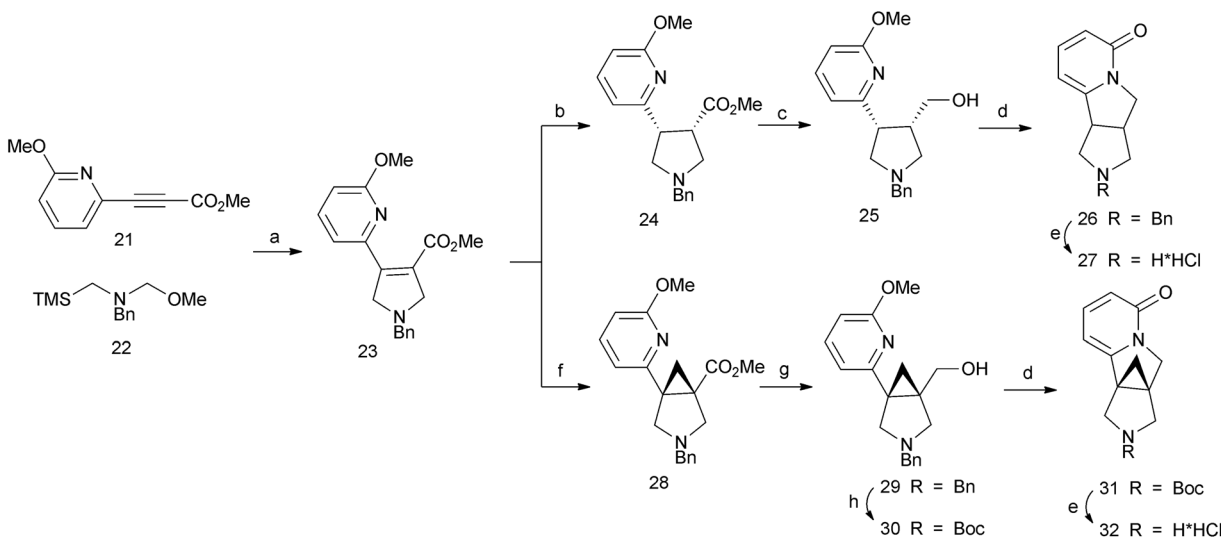
Scheme 2 Reagents and conditions: a) NaH, TBAI, allyl bromide, DMF, 0 °C to rt, 3.5 h, 97%; b) 1. H_2 (1 atm), 20% $Pd(OH)_2$–C, $(Boc)_2O$, MeOH, 72 °C, 5 min., 2. TFA, DCM, rt, 69%.



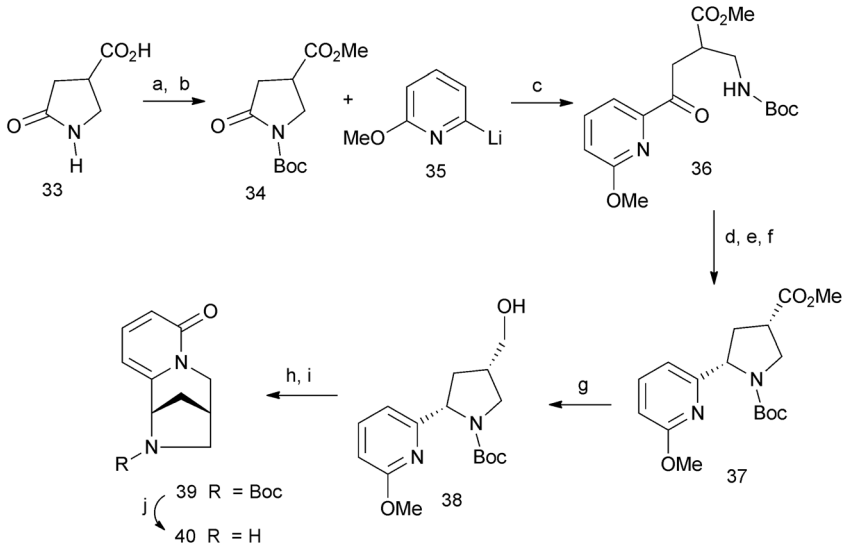
Scheme 3 Reagents and conditions: a) 1. NaH, TBAI, cyclohexylmethyl bromide or benzyl bromide, DMF, 0 °C to rt, 2. TFA, DCM, rt, (76–84%); b) 1-bromopentane or ethyl bromide, acetone, reflux, 16 h, 52–72%; c) 1. DAST, DCM, −78 °C to rt, 3 h, 2. TFA, DCM, rt, 61%.

Although the number of compounds containing the cytisine ring system is quite large, relatively little synthetic work has been done to obtain ring-contracted analogues. Two novel skeletons named cyfusine (**27**) and cyclopropylcyfusine (**32**) were synthesised originally by Yohannes.[34] The successful route commences with a [3 + 2] cycloaddition reaction between alkyne **21** and the azomethine ylid derived from amine **22** to afford the key intermediate **23** (Scheme 4). Reduction of the double bond and the ester using catalytic hydrogenation and $LiAlH_4$ gave alcohol **25**, which was cyclised using the O'Neill strategy with the mesyl group as leaving group at 0 °C followed by warming to room temperature to afford **26**. Finally, the benzyl derivative was hydrogenated in the presence of $(Boc)_2O$ and $Pd(OH)_2$ with subsequent *N*-Boc deprotection with HCl/MeOH affording cyfusine (**27**) as its hydrochloride salt. To generate cyclopropylcyfusine (**32**), addition of dimethylsulfoxonium ylide to the α,β double bond of **23** first formed **28**. Reduction of the ester (**28**) gave the alcohol (**29**), and to avoid possible problems with the removal of the benzyl group from the final tetracyclic system, this group was exchanged at this point for a Boc group using the same methodology mentioned above to give **30**. Finally, repeating the mesylation-pyridone cyclization sequence on **30**, with a similar sequence as for **25**, afforded **31** which was Boc-deproctected giving cyclopropylcyfusine (**32**) in high yield as its hydrochloride.

3-Hydroxy-11-norcytisine (**46**) is a natural product isolated fairly recently from *Laburnum anagyroides* as its bisdansyl derivative.[41] This compound was synthesized by Yohannes and co-workers who also confirmed its structure by single-crystal X-ray analysis.[35,36] In their paper, (±)-11-norcytisine (**40**) and *N*-Boc-(±)-3-amino-11-norcytisine (**42**) were synthesized as well. Their strategy was to first to access *N*-Boc-(±)-11-norcytisine (**39**) for further elaboration *via* bromination or nitration α- to the pyridone carbonyl group. To produce **39** they converted the commercial acid **33** into its methyl ester and protected the lactam nitrogen with a Boc-group (Scheme 5). Addition of 6-lithio-2-methoxypyridine to the lactam carbonyl gave the amino-ketone (**36**) in modest yield. This was *N*-Boc- deprotected with TFA and



Scheme 4 Reagents and conditions: a) TFA (0.1 equiv), CH_2Cl_2, 0 °C to rt, 2 h, 85%; b) 40 psi $H_2/Pd(OH)_2$, MeOH, 2 h, 41%; c) $LiAlH_4/Et_2O$ (1.6 equiv), Et_2O, 0 °C to rt, 1.5 h, 67%; d) MsCl (1.5 equiv), DIPEA (4 equiv), CH_2Cl_2, 0 °C, warmed to rt, 67%; e) 1. 45 psi $H_2/Pd(OH)_2$, $(Boc)_2O$, MeOH, quantitative; 2. 1 N HCl, MeOH, rt, 16 h, quantitative; f) 1 M dimethylsulfoxonium ylide in THF, THF, rt, 1.5 h, 61%; g) $LiAlH_4/Et_2O$ (1.6 equiv), Et_2O, 0 °C to rt, 1.5 h, 95%; h) 45 psi $H_2/Pd(OH)_2$, MeOH, $(Boc)_2O$, 94%; i) MsCl (1.5 equiv), DIPEA (4 equiv), CH_2Cl_2, 0 °C, warmed to rt, 16 h, 82%; j) 1 N HCl, MeOH/EtOAc/hexanes, rt, 16 h, quantitative.



Scheme 5 Reagents and conditions: a) Me_2SiCHN_2, MeOH; b) Boc_2O, Et_3N, MeCN, 73% both steps; c) THF, −78 °C, 55%; d) TFA, DCM; e) H_2/10% Pd–C, MeOH; f) Boc_2O, $NaHCO_3$, THF, 74% three steps; g) $LiAlH_4$/THF, 99%; h) MsCl, DIEA, CH_2Cl_2; i) Toluene, reflux, 84% two steps; j) TFA, DCM, 41%.

cyclised spontaneously after treatment with $NaHCO_3$ to the imine, which was hydrogenated on Pd–C to afford the pyrrolidine (**37**). The ester group was reduced and the *cis*-alcohol (**38**) was cyclised using modified van Tamelen methodology, affording the desired *N*-Boc-(±)-11-norcytisine (**39**). This compound was deprotected with TFA in dichloromethane to give (±)-11-norcytisine (**40**).

Treatment of **39** with NBS in CCl_4 provided the 3-brominated isomer **43**, which under Stille conditions gave the 3-acetyl compound **44**. This was converted to an ester and then hydrolyzed affording *N*-Boc-(±)-3-hydroxy-11-norcytisine (**45**). Deprotection gave the natural product (**46**) that was characterised as its bisdansyl (DNS) adduct (**47**) (Scheme 6). Nitration of **39** and reprotection with $(Boc)_2O$ followed by catalytic hydrogenation gave *N*-Boc-(±)-3-amino-11-norcytisine (**42**) (Scheme 6).

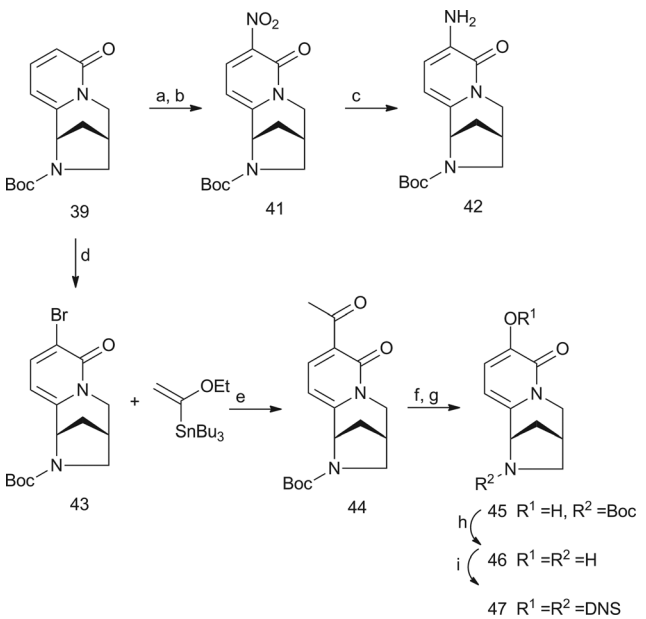
A short synthesis of 2,3,4,5-tetrahydrocytisine (**51**) was described by Scheiber and Nemes,[34] based on their earlier preparation of (±)-*N*-methyl-8-oxo-2,3,4,5-tetrahydrodesoxocytisine.[42] They began their synthesis with a double Mannich condensation of a dioxoquinolizidine (**48**) with formaldehyde and benzylamine to produce (±)-*N*-benzyl-8-oxo-2,3,4,5-tetrahydrocytisine (**49**) (Scheme 7). Both classical and modified Wolff–Kishner procedures selectively reduced the bispidine carbonyl group to afford tricyclic lactam **50**, and the benzyl group was removed by catalytic hydrogenation affording **51**.

Gallagher's group reported new cytisine syntheses in 2004 and 2006 where a key feature is the formation of the C6–C7 bond.[43,44] More recently they used this strategy to access the "uncommon" 4-halogenated cytisines. In this regard, the synthesis of the intermediate *N*-benzyl-4-fluorocytisine (**57a**) and *N*-benzyl-4-bromocytisine (**57b**) (Scheme 8a) began with *N*-alkylation of pyridones **53** with piperidinylmethyl bromide (**52**) providing lactams **54**. These were treated with LiHMDS to promote enolization and subsequent intramolecular 1,6-addition to give a 2.3 : 1 mixture of the α-6 and β-6 epimers **55**. The α-adduct was oxidised with MnO_2 to re-establish the pyridone ring (**56**) and then selective lactam reduction afforded the desired *N*-benzyl-4-halocytisine (**57**).

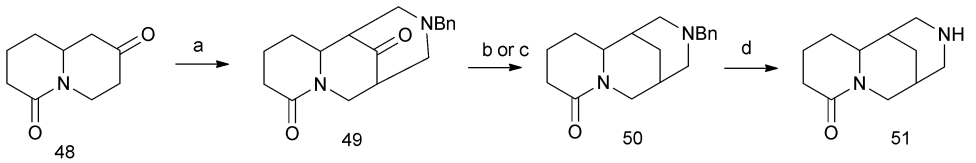
Removal of the benzyl group from **57a** gave 4 fluorocytisine (**58**) whilst treatment of (**57b**) with 1-chloroethyl chloroformate, followed by methanolysis, afforded a mixture of 4-chloro- (**59**) and 4-bromocytisine (**60**) (Scheme 8b).

The same strategy was used in this paper to synthesize cyfusine (**26**) and 4-fluorocyfusine (**26a**) (Scheme 9).[45]

Very recently, Gallagher's group reported a new cytisine synthesis using a modified van Tamelen methodology.[39] This new



Scheme 6 Reagents and conditions: a) HNO_3-H_2SO_4; b) Boc_2O, $NaHCO_3$, THF, 22% two steps; c) H_2/10% Pd–C, MeOH, 40%; d) NBS, CCl_4 54%; e) $Pd(PPh_3)_4$, toluene, reflux, 70%; f) m-CPBA, $CHCl_3$; g) KOH, MeOH, 40% two steps; h) TFA, DCM 67%; i) DNS-Cl, K_2CO_3, MeCN, 71%.



Scheme 7 Reagents and conditions: (a) CH_2O, $PhCH_2NH_2$, AcOH, MeOH, reflux 6 h, 52%; (b) $N_2H_4 \cdot H_2O$, KOH, ethylene glycol, 180 °C, 46%; (c) H_2, (1 atm), Pd–C, AcOH, 52%.

and elegant approach allowed them to develop a modular synthesis that can produce core modifications of cytisine starting from lactam **62** *via* the pivotal bromide intermediate **63**. Stille coupling between **63** and **64** formed the C6–C7 bond of cytisine, and catalytic reduction of the double bond of **65** with removal of the TBS group gave a *cis*:*trans* mixture (**66**), which was cyclisised under O'Neill's conditions to provide tricyclic lactam **67** in high yield. Reduction of **67** with borane and *N*-debenzylation afforded cytisine (Scheme 10).[33]

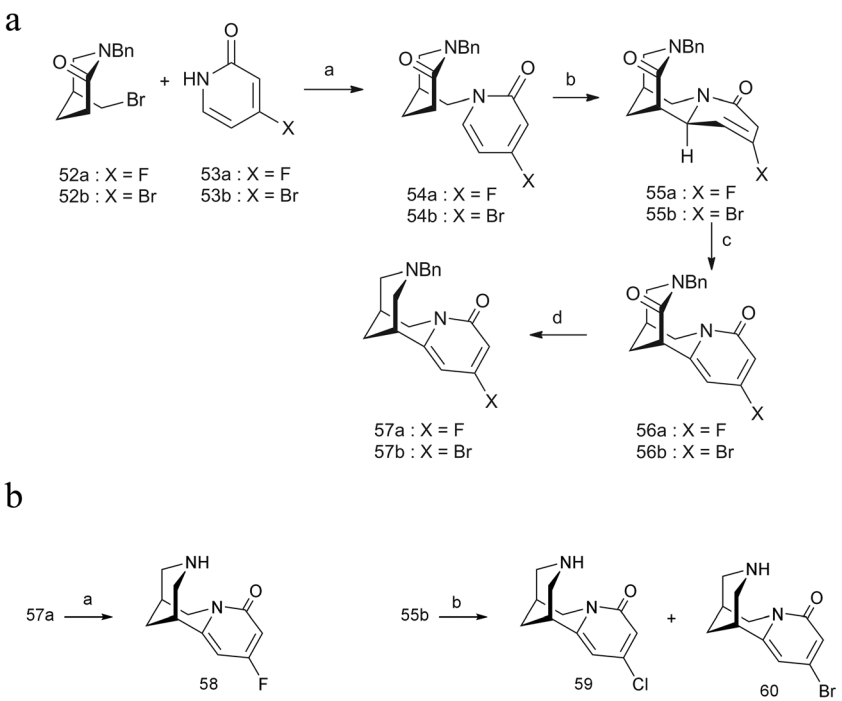
Starting from **63**, but coupling it with 2-methoxy-3-tributylstannylisoquinoline (**68**), a similar sequence led to benzo[3,4] cytisine (**72**) (Scheme 11).[33]

Finally, starting from 2-methoxy-6-tributylstannylpyrazine (**73**), 4-azacytisine (**77**) was obtained (Scheme 12).[33]

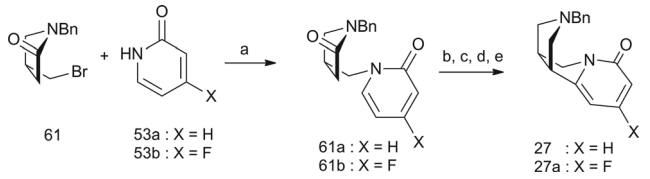
The synthesis of a cytisine/epibatidine hybrid by Rouden's group can be divided into 2 parts (Scheme 13).[46] Firstly, O'Neill's approach was followed to obtain **85**. Then, a radical cyclization was used to produce, after deprotection, 2 compounds (**87** and **89**) with nanomolar affinity at the α4β2 nAChR subtype. The initial aryl C–C coupling gave the corresponding bipyridine (**80**), which was selectively *N*-benzylated on the pyridine ring containing the ester group. Then, this pyridinium ring was reduced with $NaBH_3CN$ to give the piperidine (**82**) and the ester group of the *cis* isomer was reduced with $LiAlH_4$ affording the hydroxymethylpiperidine (**83**). The alcohol was transformed into the iodide (**84**) and the 4-methoxybenzyl group was exchanged for a methyl carbamate before cyclisation. Finally, the radical cyclization was optimised using dilauryl peroxide and $BF_3 \cdot Et_2O$ to yield the two isomeric 2-chloropyridine analogues of cytisine **70** and **72** after deprotection with 9 N HCl.

4 Pharmacology of cytisine and analogues

During most of the 20th century, pharmacological studies on (−)-cytisine and a small number of derivatives was largely limited to *in vivo* and isolated organ studies. In contrast, the proliferation of hemisynthetic and synthetic cytisinoid compounds beginning around the year 2000 occurred at a time when radioligand displacement studies had become the standard to determine affinities for a diversity of receptor subtypes. Unfortunately, different authors have used different experimental paradigms, *e.g.* radioligand displacement studies in rat brain homogenates, rat or human receptors heterologously expressed in *Xenopus* oocytes or in various cultured cell lines, *etc.* for affinity, and electrophysiological studies, stimulated neurotransmitter release or ^{86}Rb efflux for functional efficacy. Moreover, even when papers report binding studies to the same substrates, one often finds that different radioligands were used. All this makes



Scheme 8 a. Reagents and conditions: a) K_2CO_3, 73% for F and 69% for Br; b) LiHMDS, 100% for F; c) MnO_2, 100% for F and 80% for Br (two steps); d) 1. $BH_3 \cdot THF$; 2. $Pd(OH)_2$, H_2, b. Reagents and conditions: (a) H_2, $Pd(OH)_2$; (b) MeCH(Cl)OCOCl, then MeOH



Scheme 9 Reagents and conditions: a) K_2CO_3, 61% for H and 75% for F; b) LiHMDS; c) MnO_2; d) $BH_3 \cdot THF$; e) $Pd(OH)_2$, H_2, 42% for H and 14% for F (four steps).

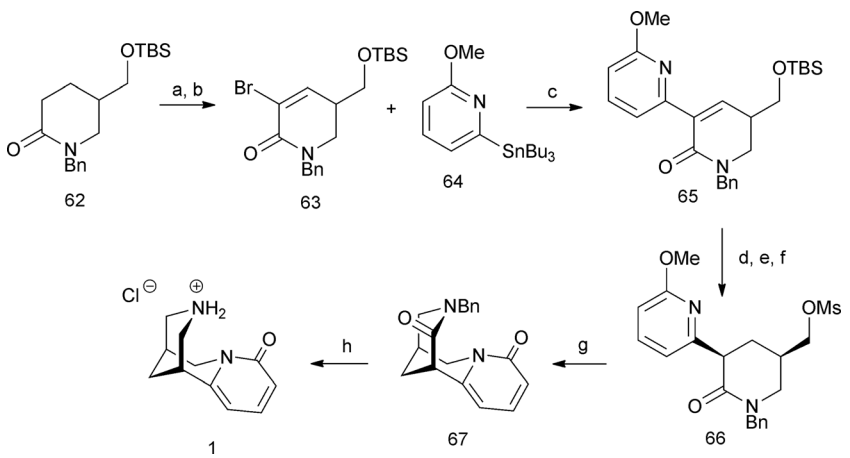
comparison of results from different laboratories, even with the same compounds, extremely difficult. For this reason we have preferred not to tabulate the sometimes very diverse data and in most cases simply describe general trends arising for the now rather large number of cytisine analogues that have been the subject of biochemical-pharmacological studies.

(−)-Cytisine was first found to resemble nicotine in its peripheral actions in 1912,[47] and this similarity was confirmed, including additional pharmacological and toxicological criteria in the following decades.[48,49] These papers showed that cytisine is similar to nicotine in its toxicity, somewhat more potent in eliciting muscle contraction, and a ganglionic stimulant also causing blockage at higher concentrations. Comparison of the crystal structures of nicotine and cytisine showed that the latter, with a 'chair' conformation of the piperidine ring as shown in Fig. 1, holds its piperidine nitrogen at about the same distance from the hydrogen bond-accepting carbonyl oxygen as that separating the pyrrolidine and pyridine nitrogens of nicotine. This is a structural feature that presumably underlies the nicotinic pharmacology of both alkaloids.[6,50] In present-day terms it must be said that cytisine is a partial agonist at nicotinic acetylcholine receptors (nAChR) containing at least combinations of the α4 and β2 subunits (α4β2* nAChR). Although no crystal structure of a nAChR with cytisine has yet been reported, Fig. 2 shows the result of a docking experiment with a model of the α4β2 nAChR. It is clear that the protonated piperidine ring nestles in an aromatic "box" including a likely cation-π interaction with tryptophan 143 and, possibly, 53.[27]

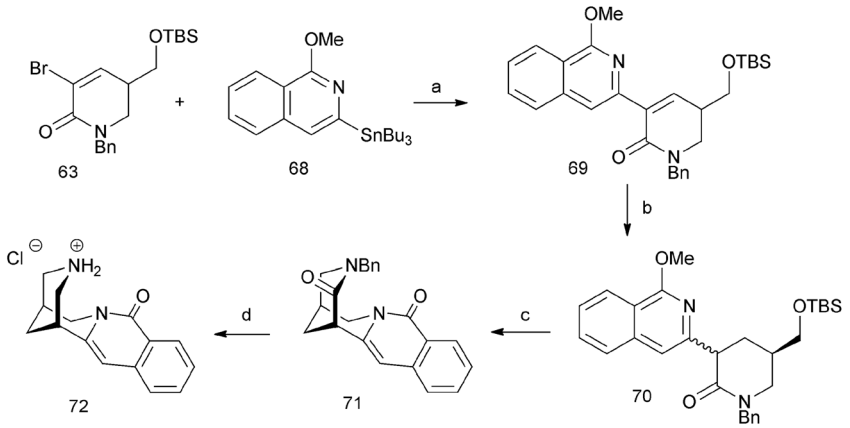
The affinity of (−)-cytisine for these receptors is greater than that of nicotine (although, being a partial agonist, *i.e.* unable to elicit a full response like the natural agonist acetylcholine, it is less efficacious). However, like this prototypical drug, it has a similar preference for central nervous system (CNS) α4β2* receptors over CNS α7 and ganglionic α3β4 nAChR, at which it is a full agonist.[25,51–63] The high affinity and selectivity of cytisine for α4β2* nAChR explains the extensive use of [^{3}H]cytisine in binding and autoradiographic studies. The nicotinic agonist activities of cytisine are probably related to its obsolete use as a respiratory analeptic,[52] and to its potent antinociceptive activity.[53,54] Interestingly, (+)-cytisine is completely devoid of affinity at the α4β2* nAChR.[44]

As discussed below (−)-cytisine is an effective aid in smoking cessation, presumably due to its potent activity as a partial agonist at α4β2* nAChR. Most recently cytisine, like nicotine, has been shown to reduce food intake by a mechanism initiated by the activation of brain α3β4 nAChR.[55] Possibly unrelated properties are the hypoglycaemic and anti-inflammatory activities claimed for cytisine and *N*-methylcytisine.[56] Cytisine also concentration-dependently reduces the formation of hydroxyl radicals in the Fenton reaction and protects mice against brain dopamine depletion caused by the dopaminergic neurotoxin MPTP.[57]

N-Methylcytisine (caulophylline) is less toxic and less potent than cytisine, and *N*,*N*-dimethylcytisinium (caulophylline methiodide) is even less so in a set of anaesthetised animal and isolated organ tests.[49] However, it appeared to be selective for nAChR from squid optical ganglia over *Torpedo marmorata* electroplax receptors.[58] Like cytisine, *N*-methylcytisine is selective for α4β2* nAChR over the other major CNS subtype (α7) and the possibly artefactual α4β4 subtype in cultured cells, but its affinity and functional potency are lower.[24,59–61] *N*,*N*-Dimethylcytisinium has only two- to sevenfold lower affinity but greater efficacy than cytisine at native rat brain receptors and ganglionic α3β4 nAChR,[60] so its very low activity *in vivo* can probably be ascribed to unfavourable pharmacokinetics. Thiocytisine has somewhat lower affinity than cytisine for α4β2*



Scheme 10 Reagents and conditions: a) 1. BuLi, PhSeCl, THF, −78 °C to 0 °C, 2. THF/MeOH/H_2O, $NaIO_4$, 14 h, 50%; b) 1. Br_2, CH_2Cl_2, 45 °C, 3 h, 2. Et_3N, CH_2Cl_2, 45 °C, 17 h, 63%; c) [$Pd(PPh_3)_4$], CuCl, LiCl, THF, 70 °C, 15 h, 99%; d) H_2, Pd/C, MeOH, 6 h; e) TBAF, THF/H_2O, 50 °C, 36 h, 85% (*cis*/*trans* 1.5 : 1.0); f) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 20 min; g) PhMe/DMF (9 : 1), 110 °C, 21 h, then LiHMDS (in THF) rt to 110 °C, 18 h, 91%; h) 1. $BH_3 \cdot THF$, 0 °C to rt, 2. $Pd(OAc)_2$, H_2 (1 atm), MeOH, 3. HCl, 60% overall from **67**.



Scheme 11 Reagents and conditions: a) $[Pd(PPh_3)_4]$, CuCl, LiCl, THF, 70 °C, 24 h, 92%; b) H_2, Pd/C, MeOH, 22 h, 100% (*cis*/*trans* 1.7 : 1.0); c) 1. $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 20 min, 2. PhMe, 110 °C, 15 h, then LiHMDS (in THF), rt to 110 °C, 24 h, 59%; d) 1. BH_3, THF, 3 h, 85%, 2. $Pd(OH)_2$, MeOH, HCl, H_2, 5 h, 68%.

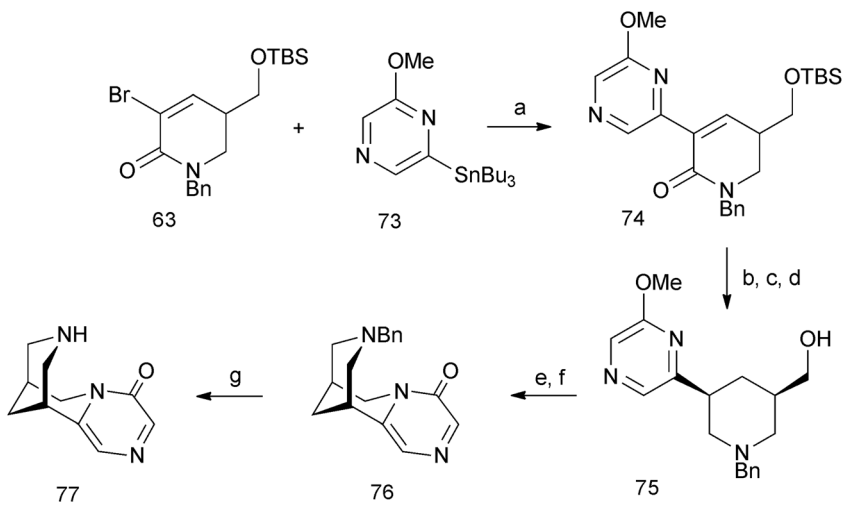
nAChR but very low affinity for α7 nAChR, making it highly selective.[24] Its efficacy at α4β2* nAChR is also somewhat lower than that of cytisine, while both affinity and efficacy at ganglionic α3β4 and neuromuscular nAChR are negligible.[60]

The preparation of *N*-2-hydroxyethylcytisine, its benzoate and cinnamate, and some aromatic esters of *N*-3-hydroxypropylcytisine was reported in 1936. Most of them "have pronounced local anaesthetic activities and… are less toxic than cocaine".[16] Several *N*-arylthiocarbamate derivatives of cytisine were tested *in vivo* as respiratory and cardiovascular stimulants. All were at least 10-20-fold less active than cytisine.[15]

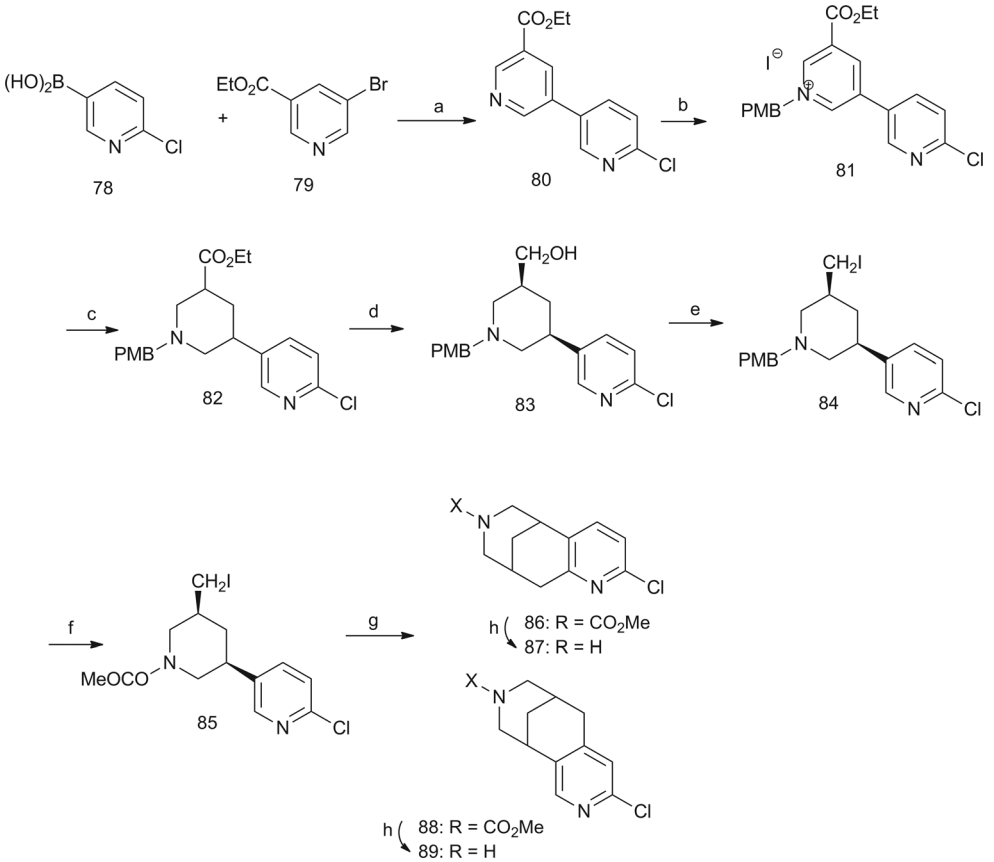
Many compounds of the extensive list of *N*-substituted (−)-cytisine derivatives prepared by Sparatore's group since 1999 have been assayed as nAChR ligands. An initial series of 31 compounds was generally disappointing regarding displacement of [^{3}H]cytisine from the nAChR of a brain membrane preparation, although 1,2-*bis*-*N*-cytisinylethane (**90**), 1,3-*bis*-*N*-cytisinylpropane (**91**), *N*-pentyl- and *N*-(3-oxobutyl)cytisine exhibited K_i values in the nanomolar range. The great majority of these 31 compounds, including those with higher nAChR affinities, were relatively nontoxic. Amongst the less toxic analogues, *N*-(3-(4-(2-methoxyphenyl)piperazin-1-ylpropyl)cytisine (**92**) exhibited potentially useful analgesic and anti-inflammatory, and modest antiallergic-antihistamine, anti-hypertensive, and hypoglycaemic activities (Fig. 3).

N-4-(4-fluorophenyl)-4-oxobutylcytisine was also anti-inflammatory and inhibited PAF-induced platelet aggregation, and in addition increased the force and decreased the rate in spontaneously beating guinea-pig right atria.[17] Interestingly, 1,2-*bis*-*N*-cytisinylethane was not only a potent antagonist of nAChR expressed by neuroblastoma cells, but also exposure to this compound (and to cytisine or nicotine) for 48 h increased the expression of α3β2* and, presumably, α7 receptors.[62]

In subsequent papers nAChR affinities were determined for a more extensive series of (−)-cytisine derivatives. Interestingly, *N*-aminocytisine was found to be almost as potent as cytisine in this assay, and 3-nitrocytisine (**93**) has twice the affinity of cytisine, while the 5-nitro derivative (**94**) was two orders of



Scheme 12 Reagents and conditions: a) $[Pd(PPh_3)_4]$, CuCl, LiCl, THF, 70 °C, 15 h, 86%; b) $LiAlH_4$, THF, 0 °C, 15 min, 89% (*cis*/*trans* 2.7 : 1.0); c) $LiAlH_4$, THF, 15 h; d) TBAF, THF/H_2O (9 : 1), 50 °C, 16 h, 49%; e) 1. $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C to rt; f) Et_3N, PhMe/DMF (4 : 1), 110 °C, 15 h, 73%; g) 1. 1-chloroethyl chloroformate, $ClCH_2CH_2Cl$, 80 °C, 17 h, 2. MeOH, 65 °C, 3 h, 61%.



Scheme 13 Reagents and conditions: a) $Pd(OAc)_2$, dppf, CsF, DME, 80 °C, 5 h, 86%; b) 4-methoxybenzyl chloride, KI, MeCN, 80 °C, 98%; c) $NaBH_3CN$, AcOH, EtOH, 20 °C, 3 h, *cis* 45%; d) $LiAlH_4$, Et_2O, 1 h, −20 °C to 0 °C, 89%; e) PPh_3, imidazole, I_2, CH_2Cl_2, 35 °C, 2 h; 78%; f) MeOCOCl, CH_2Cl_2, 20 °C, 90%; g) dilauryl peroxide, DCE, BF_3 Et_2O, 80 °C, 16 h, 37 and 15%; h) 9 N HCl, 100 °C, 16 h, 80% for **87** and 70% for **89**.

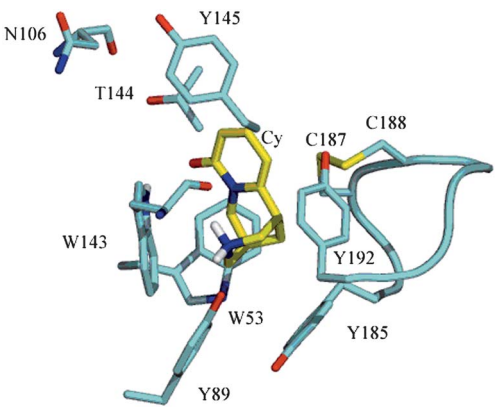


Fig. 2 A docking representation of cytisine (yellow carbon skeleton) in the active site of the human α4β2 nAChR.

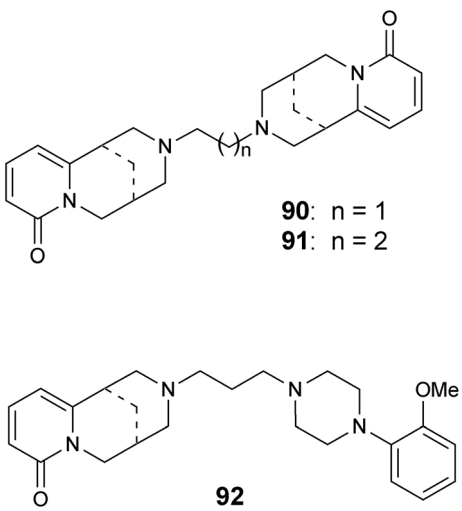


Fig. 3 Potent *N*-substituted cytisine nAChR ligands.

magnitude less potent. The affinity of tetrahydrocytisine for rat brain membranes was also about 50 times lower than that of cytisine. A CoMFA study of 20 of these compounds, after excluding the pyridone-substituted analogues and *N*-nitrosocytisine, reached the tentative conclusion that steric, rather than electrostatic interactions modulate their affinities, that longer *N*-substituents are favoured and that branching is disfavoured. This was interpreted as an explanation of the increasing pK_i values for the previously mentioned dimeric cytisines (7.02 and 7.52 for the $(CH_2)_2$ and $(CH_2)_3$ connectors, respectively) and the yet higher value (7.60) for the analogue with a CH_2CCCH_2 connector.[18,19] Nevertheless, it should be pointed out that an intrinsic weakness of 3D QSAR studies like this one is the tacit assumption that different compounds share a common binding mode. Another paper compared the binding of a subset of the former compounds to native membranes of rat brain

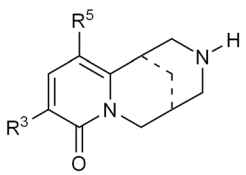
(mainly α4β2* or α7 nAChR, depending on the radioligand used) and of rat superior cervical ganglion (largely α3β4 nAChR), confirming the relatively weak affinities to α7 and ganglionic receptors. Unlike cytisine, all these compounds were nAChR antagonists or, at most, very inefficacious partial agonists. A previously unmentioned derivative, *N*-1-adamantyloxycarbonylcytisine, of interest as a possible prodrug, had very low affinity for all three nAChR subtypes.[63]

An attempt has been made to relate the crystal structures of three cytisine derivatives with bulky *N*-substituents to their widely differing affinities for α4β2* nAChR. However, the flexible substituents may adopt quite different conformations in solution and when bound to the receptor, making any conclusion rather tentative.[64] Some new compounds were recently added to this collection and assayed for α4β2* and α7 nAChR binding. In that communication a docking study for cytisine and a large set of derivatives led to the conclusion that, in addition to the generally acknowledged cation–π interaction of the protonated nitrogen atom and a hydrogen bond formed by the pyridone carbonyl, a β-carbonyl group on an *N*-substituent is generally favourable to α4β2* nAChR affinity, and that this is probably a consequence of hydrogen bonding to the hydroxyl group of a neighbouring tyrosine residue. Furthermore, an aromatic ring joined to the cytisine secondary amine nitrogen through a short connector can also establish a favourable aromatic stacking interaction.[65]

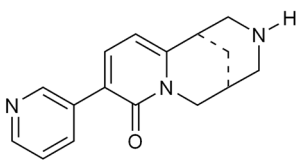
In the 1960's (−)-cytisine was introduced to the clinic as an aid to quit smoking, and it is still marketed in tablet form in some countries for this purpose (Tabex®). Several studies indicated its effectiveness,[66–68] but only very recent work conforms to present-day standards.[7] It is now clear that the chronic administration of frequent low doses of cytisine (1.5 mg orally, 6 times per day initially) is associated with higher abstinence rates than placebo, comparable to those attained with nicotine replacement therapy. However, its unfavourable pharmacokinetics seem to be an important limitation to its use.,[69–71] The discriminative stimulus effects in rats of cytisine have been studied on several occasions, indicating partial generalization to nicotine.[72–79] Varenicline, a smoking cessation drug designed on the basis of the cytisine structure and, like the natural product, a partial agonist mainly at α4β2* nAChR, has been directly compared with cytisine in this regard, and shown to have similar, but stronger effects.[79] Like nicotine, cytisine elicits the release of dopamine (and, with much lower potency, noradrenaline) from brain tissue, both *in vitro* and *in vivo*.[61] This effect is believed to underlie the addictive properties of nicotine and is presumably related to the increased locomotor activity elicited by nicotine and cytisine in laboratory animals.[80,81]

(−)-Cytisine exhibits antidepressant-like effects in several murine models of depression when injected at doses of 1.5 mg kg^{-1}, and reduces c-fos immunoreactivity in the basolateral amygdala, a brain structure where similar changes are elicited by classical antidepressants. These effects were tentatively attributed to the partial agonist character of cytisine at α4β2* nAChR, as some similar results had been obtained with nicotine (which inactivates nAChR upon chronic administration) and the nonselective antagonist mecamylamine.[82] 3-(3′-Pyridyl)cytisine (**95**) (Fig. 4), which has very low efficacy at α4β2* nAChR and causes no measurable response at other subtypes, also showed dose-dependent antidepressant-like effects. Peripheral administration of the low efficacy partial agonist 5-bromocytisine did not elicit any antidepressant-like behaviours, although it was effective in one model when administered intracerebrally, suggesting that its inactivity might be related to poor access to the brain.[83]

Binding studies have been done on the easily accessible 3- (**96**) and 5- (**97**) chloro-, bromo- and iodocytisines and the corresponding dihalo (**98**) compounds (Fig. 4), using native and recombinant, rat and human nAChR and different radioligands, which may explain in part the quantitatively different results. Nevertheless, halogenation at C3 consistently results in increased affinity for α4β2 and α4β2* nAChR but also for the α7 subtype, with some loss of selectivity. Similar affinity enhancements were found at α4β3 and α4β4 nAChR. Different functional assays (electrophysiological, calcium entry, membrane potential, neurotransmitter release) generally showed that the 3-halo derivatives are also more potent than cytisine, with little change in their efficacies, *i.e.* all are partial agonists. Halogenation at C5 or at C3 and C5 leads to reduced affinity and functional potency.[24,25,59–61] As stated above, 3-nitrocytisine has high affinity for brain membranes, and 5-nitrocytisine is much less potent.[19] Functional experiments demonstrated that 3-nitrocytisine is a very low efficacy partial agonist at α4β2 receptors, with potency in the micromolar range. At α7 receptors, it is a full agonist with higher functional potency than 3-bromocytisine. 5-Nitrocytisine showed no activity at either nAChR subtype. The affinity profile of the recently prepared 3-bromo-5-nitrocytisine resembles that of 3,5-dibromocytisine, and this derivative is a partial agonist with very low efficacy at α4β2 receptors and a full agonist of higher functional potency than 3,5-dibromocytisine. On the other hand, 5-bromo-3-nitrocytisine has low affinity at α4β2 nAChR, does not bind appreciably to the α7 subtype, and is functionally inactive at both subtypes.[27] Bromination of *N*-methylcytisine at C3 raised affinity for α4β2 nAChR to approximately the same level as cytisine and had a smaller effect at α7 receptors. Bromination at C5 was strongly detrimental to α4β2 receptor affinity and less so to α7 nAChR binding. Both the 3-bromo and the 5-bromo derivative are partial agonists at α4β2 nAChR.[59,61] 3-Methyl-, 3-trifluoromethyl- and 3-fluorocytisine have been synthesised and assayed more recently. The methyl and trifluoromethyl derivatives showed increased affinity for α4β2* nAChr, approaching



93: $R^3 = NO_2$; $R^5 = H$
94: $R^3 = H$; $R^5 = NO_2$
96: $R^3 = Hal$; $R^5 = H$
97: $R^3 = H$; $R^5 = Hal$
98: $R^3 = Hal$; $R^5 = Hal$



95

Fig. 4 Potent 3- and 5-substituted cytisine nAChR ligands.

the value for 3-bromocytisine, but their low affinities for the α7 subtype are similar to that of cytisine, and they are therefore several times more selective than the natural product. Unexpectedly, the α4β2* affinity of 3-fluorocytisine is about six times lower than that of cytisine.[26]

The first *in vivo* study of the potent halogenated (−)-cytisine derivatives followed on the demonstration of the dopamine-releasing effects of these compounds in rat brain slices, in which 3-bromo- and 3-iodocytisine were ten or more times as potent as cytisine and nicotine as releasers of dopamine and norepinephrine from brain slices.[61] Microdialysis in anaesthetised rats, with the probe placed in the corpus striatum and introducing the drugs through the microdialysis cannula, showed that dopamine release elicited by nicotine, cytisine and 5-bromocytisine was dose-dependent with the apparent rank order of potency 5-bromocytisine > nicotine > cytisine. The effect of 3-bromocytisine exhibited little dose-dependency and at the highest concentration assayed (10 mM) it released less dopamine than 10 mM cytisine but, unlike the other drugs, its effect was measurable at a concentration 10 times lower than the threshold concentration of cytisine, nicotine or 5-bromonicotine. In a rat model of Parkinson's disease (caused by injection of 6-hydroxydopamine into the striatum), subcutaneous nicotine, cytisine (at twice the dose) and 5-bromocytisine (at the same dose as nicotine) attenuated the decrease in striatal dopamine levels. The *in vitro* less efficacious 3-bromocytisine, at a dose 10 times lower than 5-bromocytisine (0.1 mg kg^{-1}, as higher doses were not tolerated) had no effect in spite of its higher *in vitro* potency.[61,84] After acute administration of nicotine, cytisine, 3- or 5-bromocytisine, only 3-bromocytisine (at a dose level 10 times lower than that of the other drugs tested) significantly increased locomotion once the animals were habituated to the novel test environment, but reduced rearings, generally associated with exploratory activity. These effects were shown to be mediated by nAChR in the striatum, and to involve the dopaminergic system.[85]

(−)-Cytisine and its 3- and 5-bromo derivatives were used as tools to characterise the nAChR in cat petrosal ganglion neurons in culture. The relative potencies of different agonists suggested a predominance of the α7 subtype in these cells.[86] 3-Iodocytisine was studied in greater detail in live mice. Intraperitoneal injection of this compound produced a profound, reversible hypothermia (a drop of up to 13 °C in body temperature at 1 mg kg^{-1}), an effect that was related to interaction mainly with β4 (and to a lesser extent β2) subunit-containing nAChR in both the central and peripheral nervous systems. Lower doses (0.2 mg kg^{-1}) were well tolerated and also produced significant, reproducible hypothermia. This level was therefore used to study the up-regulation of α4β2* and α7 nAChR (β4* nAChR were insensitive) in different brain regions, which was found to be greater than that elicited by nicotine.[87]

(−)-3-Vinylcytisine had somewhat better affinity than (−)-cytisine for all the nAChR subtypes tested (α2β2, α2β4, α3β2, α3β4, α4β2, α4β4), showing a similar moderate selectivity (130-fold) for the abundant brain α4β2 *vs.* the important ganglionic α3β4 subtype. Substitution at C3 with an aryl group led to sharply reduced affinities for all these nAChR subtypes, particularly with the very bulky 4-*n*-butylphenyl group.[29]

(±)-4-Methylcytisine has practically the same affinity for α4β2 nAChR as (−)-cytisine, but its affinities for all other subtypes tested (α2β2, α2β4, α3β2, α3β4, α4β4) were considerably lower, suggesting a possibly useful selectivity, particularly *vs.* the ganglionic α3β4 subtype (3526-fold). Its agonist potency at α4β2 nAChR was about 7 times greater than that of the full agonist nicotine, but with only 22% efficacy, whilst a similar comparison at the α3β4 subtype revealed almost identical potency as nicotine and 83% efficacy. (±)-4-Hydroxymethylcytisine bound more weakly to all these receptors than (±)-4-methylcytisine, but still retaining 909-fold α3β4/α4β2 selectivity.[29] (±)-4-Fluoromethylcytisine resembled the non-fluorinated analogue in its affinities. Substitution on the hydroxymethyl oxygen of (±)-4-hydroxymethylcytisine (propyl, cyclohexylmethyl, benzyl, trifluoromethylbenzyl, fluorobenzyl) was generally detrimental to binding, although the cyclohexylmethyl and benzyl ethers were more selective than cytisine, and the latter two still exhibited low nanomolar affinities and even higher selectivities than (±)-4-methylcytisine. Alkylation (ethyl or *n*-pentyl) of the piperidine nitrogen of these two ethers led to the least potent and least selective members of the series.[38] Two (−)-cytisine derivatives substituted at C10 (C6 in the IUPAC numbering): 10-α-methoxycarbonyl- and 10-α-propionylcytisine, had negligible affinities for all nAChR subtypes tested.[29]

Amongst Gallagher's 'core-modified' cytisine analogues, only benzo[3,4]cytisine (**84**), which can be viewed as a 3,4-disubstituted cytisine, seems to have been subjected to a binding study that indicated that this compound has fairly high affinity (IC_{50} = 85 nM) for [^{3}H]epibatidine-labeled whole rat brain membranes, which probably represents mainly α4β2* nAChR binding.[88] The ring-contracted 11-norcytisine and 3-hydroxy-11-norcytisine have rather low nAChR affinities, in the micromolar range for the α4β2 and above 10^{-4} M for the α7 subtype, respectively.[36] This suggests that the preferred geometry of cytisine, with the basic nitrogen atom lying almost above the pyridone ring (Fig. 1), is crucial for nicotinic receptor binding.

A large number of *carba*-analogues of cytisine (with a variously substituted benzene ring instead of the pyridone ring) have been synthesised and assayed for affinity, mainly at α4β2 but in some low- to subnanomolar affinity cases at other nAChR. All exhibited lower affinities than cytisine at this receptor subtype (in several cases K_i > 500 nM), but the presence of a small electron-withdrawing group favoured affinity. The three most potent compounds of this series (3-methoxy, 3-fluoro and 2,3-difluoro, numbered congruently with the usual cytisine numbering) were tested and compared with cytisine for their effects on spontaneous and nicotine-elicited dopamine turnover in rat nucleus accumbens: the 3-methoxy analogue strongly resembled cytisine in this regard, eliciting dopamine release but partially blocking the releasing effect of nicotine, while the fluorinated derivatives had no significant effect on spontaneous release but were more effective than cytisine in reducing the nicotine response.[37,89]

5 Perspectives

(−)-Cytisine, its derivatives and analogues are of interest as pharmacological tools and as potential drugs for the treatment of a wide variety of conditions, from eating disorders, nicotine and alcohol dependence, to depression, schizophrenia and neurodegenerative diseases. (−)-Cytisine itself is used as an aid to quit tobacco smoking, although it is not very effective and

appropriate structural modification might well make it more so. The only related compound in current therapeutic use, conceptually derived from cytisine though not strictly a cytisine derivative or analogue, is the smoking cessation drug varenicline (Chantix®, Champix®).[89] Several publications reviewed here suggest that some cytisinoids show promise as appetite reducers, antidepressants or drugs to treat Parkinson's disease.

Relatively few natural products are used as such in modern medicine. In contrast, many drugs are modified natural products or have been developed from natural product templates. Natural products have an important place in the history of medicinal chemistry, and their structural modification to modulate their potency and selectivity or promiscuity, to improve their solubility or their pharmacokinetics, continue to be an intellectually challenging and potentially rewarding field. In this regard, cytisine has been used as a model compound for little more than a decade, with remarkable commercial success by the Pfizer group and with unanticipated and promising activities arising from work carried out largely in Europe and South America. Much remains to be done, and we firmly believe that active research into the chemistry and pharmacology of cytisine and cytisinoids will not only continue to generate basic knowledge but is also likely to provide us with novel drugs for pathologies that are often refractory to treatment or for which current therapies are unsatisfactory.

6 Acknowledgements

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7 References

1 A. Husemann and W. Marmé, *Z. Chemie*, 1865, 161.
2 H. R. Ing, *J. Chem. Soc.*, 1932, 2778.
3 S. Okuda, K. Tsuda and H. Kataoka, *Chem. Ind. (London)*, 1961, 1751.
4 A. A. Freer, D. J. Robins and G. N. Sheldrake, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1987, **C43**, 1119.
5 P. Mascagni, M. Christodoulou, W. A. Gibbons, K. Asres, J. D. Phillipson, N. Niccolai and S. Mangani, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1159.
6 R. B. Barlow and O. Johnson, *Br. J. Pharmacol.*, 1989, **98**, 799.
7 R. West, W. Zatoński, M. Cedzynska, D. Lewandowska, J. Pazik, P. Aveyard and J. Stapleton, *N. Engl. J. Med.*, 2011, **365**, 1193.
8 K. Buchka and A. Magalhães, *Ber. Dtsch. Chem. Ges.*, 1891, **24**, 697.
9 A. Partheil, *Arch. Pharm.*, 1892, **230**, 448.
10 A. Partheil, *Arch. Pharm.*, 1894, **232**, 486.
11 J. Lammers, *Arch. Pharm.*, 1897, **235**, 374.
12 M. Freund and A. Friedmann, *Ber. Dtsch. Chem. Ges.*, 1901, **34**, 605.
13 M. Freund and P. Horkheimer, *Ber. Dtsch. Chem. Ges.*, 1906, **39**, 814.
14 F. Litterscheid, *Arch. Pharm.*, 1900, **238**, 191.
15 G. Luputiu and L. Gilau, *Arch. Pharm.*, 1969, **302**, 943.
16 H. R. Ing and R. P. Patel, *J. Chem. Soc.*, 1936, 1774.
17 C. Canu Boido and F. Sparatore, *Farmaco*, 1999, **54**, 438.
18 O. Nicolotti, C. Canu Boido, F. Sparatore and A. Carotti, *Farmaco*, 2002, **57**, 469.
19 C. Canu Boido, B. Tasso, V. Boido and F. Sparatore, *Farmaco*, 2003, **58**, 265.
20 G. Luputiu and F. Moll, *Arch. Pharm.*, 1971, **304**, 151.
21 A. Orjales, L. Ribas and A. Varela, *An. Quím.*, 1972, **68**, 1419.
22 E. Marrière, J. Rouden, V. Tadino and M.-C. Lasne, *Org. Lett.*, 2000, **2**, 1121.
23 A. Galdámez, M. Gutiérrez-Hernández, B. K. Cassels and P. Sáez-Briones, *J. Chil. Chem. Soc.*, 2011, **56**, 595.
24 P. Imming, P. Klaperski, M. T. Stubbs, G. Seitz and D. Gündisch, *Eur. J. Med. Chem.*, 2001, **36**, 375.
25 L. M. Houlihan, Y. Slater, D. L. Guerra, J.-H. Peng, J.-P. Kuo, R. Lukas, B. K. Cassels and I. Bermúdez, *J. Neurochem.*, 2001, **78**, 1029.
26 N. Houllier, J. M. Gopisetti, P. Lestage, M.-C. Lasne and J. Rouden, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6667.
27 P. Sáez-Briones, M. Rebolledo-Fuentes, A. Carbone, M. Moroni, G. Zapata-Torres, M. Gutiérrez-Hernández, P. C. Biggin, B. K. Cassels and I. Bermúdez, unpublished work.
28 G. Roger, B. Lagnel, J. Rouden, L. Besret, H. Valette, S. Demphel, J. M. Gopisetti, C. Coulon, M. Ottaviani, L. A. Wrenn, S. R. Letchworth, G. A. Bohme, J. Benavides, M.-C. Lasne, M. Bottlaender and F. Dollé, *Bioorg. Med. Chem.*, 2003, **11**, 5333.
29 S. K. Chellappan, Y. Xiao, W. Tueckmantel, K. J. Kellar and A. P. Kozikowski, *J. Med. Chem.*, 2006, **49**, 2673.
30 J. Rouden, A. Ragot, S. Gouault, D. Cahard, J.-C. Plaquevent and M.-C. Lasne, *Tetrahedron: Asymmetry*, 2002, **13**, 1299.
31 N. Houllier, S. Gouault, M.-C. Lasne and J. Rouden, *Tetrahedron*, 2006, **62**, 11679.
32 D. Stead and P. O'Brien, *Tetrahedron*, 2007, **63**, 1885.
33 C. Hirschhäuser, C. A. Haseler and T. Gallagher, *Angew. Chem., Int. Ed.*, 2011, **50**, 5162.
34 P. Scheiber and P. Nemes, *Arkivoc (iii)*, 2008, 194.
35 D. Yohannes, K. Procko, L. A. Lebel, C. B. Fox and B. T. O'Neill, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2316.
36 D. Yohannes, C. P. Hansen, S. R. Akireddy, T. A. Hauser, M. N. Kiser, N. J. Gurnon, C. S. Day, B. Bhatti and W. S. Caldwell, *Org. Lett.*, 2008, **10**, 5353.
37 J. W. Coe, M. G. Vetelino, C. G. Bashore, M. W. Wirtz, P. R. Brooks, E. P. Arnold, L. A. Lebel, C. B. Fox, S. B. Sands, T. I. Davis, D. W. Schulz, H. Rollema, F. D. TingleyIII and B. T. O'Neill, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 2974.
38 A. P. Kozikowski, S. K. Chellappan, Y. Xiao, K. M. Bajjuri, H. Yuan, K. J. Kellar and P. A. Petukhov, *ChemMedChem*, 2007, **2**, 1157.
39 B. T. O'Neill, D. Yohannes, M. W. Bundesmann and E. P. Arnold, *Org. Lett.*, 2000, **2**, 4201.
40 E. E. van Tamelen and J. S. Baran, *J. Am. Chem. Soc.*, 1955, **77**, 4944.
41 A. R. Hayman and D. O. Gray, *Phytochemistry*, 1989, **28**, 673.
42 P. Scheiber and P. Nemes, *Liebigs Ann.*, 1994, 1033.
43 C. Botuha, C. M. S. Galley and T. Gallagher, *Org. Biomol. Chem.*, 2004, **2**, 1825.
44 D. Gray and T. Gallagher, *Angew. Chem., Int. Ed.*, 2006, **45**, 2419.
45 P. Durkin, P. Magrone, S. Matthews, C. Dallanoce and T. Gallagher, *Synlett*, 2010, 2789.
46 N. Houllier, M.-C. Lasne, R. Bureau, P. Lestage and J. Rouden, *Tetrahedron*, 2010, **66**, 9231.
47 H. H. Dale and P. P. Laidlaw, *J. Pharmacol. Exp. Ther.*, 1912, **3**, 205.
48 J. Zachowski, *Arch. Exp. Path. Pharmak.*, 1938, **189**, 327.
49 R. B. Barlow and L. J. McLeod, *Br. J. Pharmacol.*, 1969, **35**, 161.
50 R. P. Sheridan, R. Nilakantan, J. S. Dixon and R. Venkataraghavan, *J. Med. Chem.*, 1986, **29**, 899.
51 L. A. Pabreza, S. Dhawan and K. J. Kellar, *Mol. Pharmacol.*, 1991, **39**, 9.
52 M. J. Dallemagne and C. Heymans, Respiratory stimulants, in *The Alkaloids*, ed. R. H. F. Manske, Academic Press, New York, 1955, Vol. 109.
53 T. S. Rao, L. D. Correa, R. T. Reid and G. K. Lloyd, *Neuropharmacology*, 1996, **35**, 393.
54 T. W. Seale, S. Singh and G. Basmadjan, *NeuroReport*, 1998, **9**, 201.
55 Y. S. Mineur, A. Abizaid, Y. Rao, R. Salas, R. J. DiLeone, D. Gündisch, S. Diano, M. De Biasi, T. L. Horvath, X.-B. Gao and M. B. Picciotto, *Science*, 2011, **332**, 1330.
56 I. Murakoshi, Y. Fuji, S. Takedo and J. Arai. Japanese Patent 04-295480 (20-10-1992); *Chem. Abstr.*, 1992, **118**, 45733; I. Murakoshi, Y. Fuji, H. Kawamura and H. Maruyama. Japanese Patent 04-295479 (20-10-1992); *Chem. Abstr.*, 1992, **118**, 45734.
57 B. Ferger, C. Spratt, P. Teismann, G. Seitz and K. Kuschinsky, *Eur. J. Pharmacol.*, 1998, **360**, 155.
58 G. IuPliashkevich and V. P. Demushkin, *Bull. Exp. Biol. Med.*, 1987, **104**, 690.
59 Y. E. Slater, L. M. Houlihan, P. D. Maskell, R. Exley, I. Bermúdez, R. J. Lukas, A. C. Valdivia and B. K. Cassels, *Neuropharmacology*, 2003, **44**, 503.

60 R. W. Fitch, Y. Kaneko, P. Klaperski, J. W. Daly, G. Seitz and D. Gündisch, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1221.
61 J. A. Abin-Carriquiry, M. H. Voutilainen, J. Barik, B. K. Cassels, P. Iturriaga-Vásquez, I. Bermúdez, C. Durand, F. Dajas and S. Wonnacott, *Eur. J. Pharmacol.*, 2006, **536**, 1.
62 L. Riganti, C. Matteoni, S. Di Angelantonio, A. Nistri, A. Gaimarri, F. Sparatore, C. Canu-Boido, F. Clementi and C. Gotti, *Br. J. Pharmacol.*, 2005, **146**, 1096.
63 E. Carbonnelle, F. Sparatore, C. Canu-Boido, C. Salvagno, B. Baldani-Guerra, G. Terstappen, R. Zwart, H. Vijverberg, F. Clementi and C. Gotti, *Eur. J. Pharmacol.*, 2003, **471**, 85.
64 G. Bombieri, F. Meneghetti, R. Artali, B. Tasso, C. Canu Boido and F. Sparatore, *Chem. Biodiversity*, 2008, **5**, 1867.
65 B. Tasso, C. Canu Boido, E. Terranova, C. Gotti, L. Riganti, F. Clementi, R. Artali, G. Bombieri, F. Meneghetti and F. Sparatore, *J. Med. Chem.*, 2009, **52**, 4345.
66 P. Tutka and W. Zatoński, *Pharmacol. Rep.*, 2005, **58**, 777.
67 J.-F. Etter, *Arch. Intern. Med.*, 2006, **166**, 1553.
68 V. Tzankova and N. Danchev, *Biotechnol.Biotechnol. Equip.*, 2007, **21**, 151.
69 H. P. Klocking, M. Richter and G. Damm, *Arch Toxicol. Suppl.*, 1980, **4**, 312.
70 H. Astroug, R. Simeonova, L. V. Kassabova, N. Danchev and D. Svinarov, *Interdisc. Toxicol.*, 2010, **3**, 15.
71 H. Rollema, A. Shrikhande, K. M. Ward, F. D. Tingley 3rd, J. W. Coe, B. T. O'Neill, E. Tseng, E. Q. Wang, R. J. Mather, R. S. Hurst, K. E. Williams, M. de Vries, T. Cremers, S. Bertrand and D. Bertrand, *Br. J. Pharmacol.*, 2010, **160**, 334.
72 J. A. Pratt, I. P. Stolerman, H. S. Garcha, V. Giardini and C. Feyerabend, *Psychopharmacology*, 1983, **81**, 54.
73 R. M. Craft and J. L. Howard, *Psychopharmacology*, 1988, **96**, 281.
74 C. Reavill, B. Walther, I. P. Stolerman and B. Testa, *Neuropharmacology*, 1990, **29**, 619.
75 J. D. Brioni, D. J. Kim, A. B. O'Neill, J. E. Williams and M. W. Decker, *Brain Res.*, 1994, **643**, 1.
76 I. P. Stolerman, H. S. Garcha, J. A. Pratt and R. Kumar, *Psychopharmacology*, 1984, **84**, 413.
77 C. J. Chandler and I. P. Stolerman, *Psychopharmacology*, 1997, **129**, 257.
78 J. W. Smith, A. Mogg, E. Tafi, E. Peacey, I. A. Pullar, P. Szekeres and M. Tricklebank, *Psychopharmacology*, 2007, **190**, 157.
79 M. G. LeSage, D. Shelley, J. T. Ross, F. I. Carroll and W. A. Corrigall, *Pharmacol., Biochem. Behav.*, 2009, **91**, 461.
80 E. Museo and R. A. Wise, *Pharmacol., Biochem. Behav.*, 1994, **48**, 521.
81 E. Museo and R. A. Wise, *Brain Res.*, 1995, **670**, 257.
82 Y. S. Mineur, O. Somenzi and M. B. Picciotto, *Neuropharmacology*, 2007, **52**, 1256.
83 Y. S. Mineur, C. Eibl, G. Young, C. Kochevar, R. L. Papke, D. Gündisch and M. B. Picciotto, *J. Pharmacol. Exp. Ther.*, 2009, **329**, 377.
84 J. A. Abin-Carriquiry, G. Costa, J. Urbanavicius, B. K. Cassels, M. Rebolledo-Fuentes, S. Wonnacott and F. Dajas, *Eur. J. Pharmacol.*, 2008, **589**, 80.
85 J. A. Abin-Carriquiry, J. Urbanavicius, C. Scorza, M. Rebolledo-Fuentes, S. Wonnacott, B. K. Cassels and F. Dajas, *Eur. J. Pharmacol.*, 2010, **634**, 89.
86 R. Varas, V. Valdés, P. Iturriaga-Vásquez, B. K. Cassels, R. Iturriaga and J. Alcayaga, *Brain Res.*, 2006, **1072**, 72.
87 C. A. Zambrano, M. J. Marks, B. K. Cassels and R. B. Maccioni, *Neuropharmacology*, 2009, **57**, 332.
88 A. Kennett, S. Wonnacott, C. Hirschhäuser, C. A. Haseler and T. Gallagher, unpublished results, cited in Hirschhäuser *et al.*, 2011 (ref 33).
89 J. W. Coe, H. Rollema and B. T. O'Neill, *Annu. Rep. Med. Chem.*, 2009, **44**, 71.