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# Halogenated and isosteric cytisine derivatives with increased affinity and functional activity at nicotinic acetylcholine receptors

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**Abstract**—A series of pyridone ring-modified derivatives of (7*R*,9*S*)-(−)-cytisine were evaluated for affinity and functional activity at neuromuscular α1β1γδ, ganglionic α3β4, and central neuronal α4β2 subtypes of nicotinic receptors. Halogenation at the 3-position improved affinity and functional activity, while substitution at the 5-position led to modest decreases in both, and disubstitution led to near abolition of functional activities and could be correlated with the electron-withdrawing ability of the halogen. Subtype selectivities of the halogenated derivatives were altered relative to cytisine in a substitution-dependent manner. Caulophylline methiodide was less potent than cytisine, but retained significant activity. Thiocytisine was relatively weak in potency and efficacy, but was significantly selective for the α4β2 subtype.

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(−)-Cytisine (**1**) is a quinolizidine alkaloid isolated from the seeds of *Cytisus* and related members of Leguminosae (Fabaceae). It is a nicotinic acetylcholine receptor partial agonist with high affinity for the central neuronal α4β2 subtype, which is one of two major receptor subtypes in brain.[1,2] Its affinity is sufficiently high to have allowed development of a radioligand with substantial utility in labeling nicotinic receptors in rat and human brain.[3] While this high-affinity agonist has been known for many years, being isolated in the mid 1800's, its structure was not determined until the 1930's.[4,5] The delineation of structure–activity relationships for cytisine has been somewhat limited, owing to its complex tricyclic structure, which presents a substantial synthetic challenge.[6–11] Indeed, cytisine has been synthesized only recently in enantiopure form.[12] Until recently, analogs have been limited to those easily obtained from the natural material and are limited to substitution on the aliphatic nitrogen and pyridone ring.

In the current study, a series of halogenated derivatives of cytisine were evaluated for functional activity in cultured cells expressing three major types of nicotinic receptors. Prior studies have focused on affinities in binding assays.[6] Cell lines expressing subunit combinations corresponding to neuromuscular receptors (TE-671)[13] and central neuronal α4β2 receptors (K-177)[14] were used to evaluate functional activity using a fluorescent membrane potential sensitive assay.[15] KXα3β4R2 cells[16] expressing ganglionic-type α3 and β4 subunits were used to assess affinity and functional activity, using $[^3H]$-epibatidine binding[17] and fluorescent calcium dynamics assays, respectively.[15,18]

Cytisine derivatives were prepared as previously described (Fig. 1).[6] Electrophilic halogenations were performed on cytisine acetate using *N*-chlorosuccinimide, *N*-bromosuccinimide, and iodine monochloride for introduction of halogen substituents, resulting in production of the 3- and 5-halo derivatives as well as the 3,5-dihalo derivatives, which were separated by chromatography and converted to their fumaric acid salts.

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*Keywords*: Nicotinic receptors; Cytisine; Acetylcholine; Structure–activity relationships.

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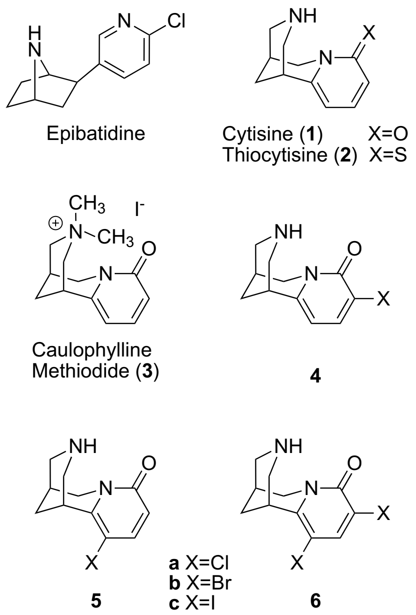

**Figure 1.** Compounds used in this study.

Thiocytisine was prepared from the parent compound by the action of Lawesson's reagent under microwave irradiation.

Biological evaluation of the compounds was conducted with cultured cells expressing native and transfected subunit combinations, including α1β1γδ (TE-671), α4β2 receptors (K-177), and α3β4 receptors (KXα3β4R2). The affinities of the cytisines for membranes from KXα3β4R2 cells were determined by $[^3H]$-epibatidine (0.5 nM) binding in a standard filtration assay format.[18] Functional agonist activity of the cytisines in the above-mentioned cell lines were determined in KXα3β4R2 cells using a fluorescence assay sensitive to changes in cytosolic calcium.[15,18] Activity in TE-671 and K-177 cells were conducted using a fluorescence assay sensitive to changes in membrane potential.[15] The latter assay was used in these cells due to their low to absent signals in the calcium assay. $EC_{50}$ values in this assay have been shown to be somewhat greater in potency as compared to the calcium assay.[15] Results of biological evaluation are summarized in Table 1 and Figure 2.

As observed previously,[6–8] halogenation at the 3-position of cytisine (**4a–c**) afforded greater potency in both binding and functional assays, with a preference for the less electronegative and bulkier iodide. In the 3-halogenated series, there was an increase in the selectivity for the α4β2 subtype as well as increases in efficacy for α4β2 and α3β4 subtypes. Neuromuscular potencies and efficacies were not greatly affected for this series.

Halogenation at the 5-position (**5a**,**b**) afforded less potent compounds, with the less electronegative bromide having higher potency. Insufficient 5-iodocytisine was available for this study. The 5-halo compounds lost virtually all activity at the neuromuscular subtype and only the 5-bromo compound retained activity at the α3β4 subtype, although both potency and efficacy were markedly reduced.

Dihalogenation (**6a–c**) afforded inactive compounds except in the case of the 3,5-diiodo derivative (**6c**), which still lost 2 orders of magnitude in potency at the α4β2 subtype. The diiodo compound lost all activity in the other two subtypes, demonstrating increased selectivity, but at too low a potency to be of great utility.

Of note, some disagreement exists between the bromocytisine affinity data obtained in rat brain[6] versus that in oocytes[7,8] expressing human neuronal α4β2

**Table 1.** Affinities and functional activities of compounds in this study

| Nicotinic agent | KXα3β4R2 rat α3β4 | | Rat brain (Primarily α4β2) | K-177 Human α4β2 | TE-671 Human α1β1γδ |
|---|---|---|---|---|---|
| | Affinity[a] | Calcium dynamics[c] | Affinity[a,b] | Membrane potential[c] | |
| (±)-Epibatidine | 0.51 ± 0.04 | 0.037 ± 0.013 (100) | 0.0072 ± 0.0005[d] | 0.0088 ± 0.0017 (100) | 0.053 ± 0.016 (100) |
| (−)-Cytisine **1** | 220 ± 10 | 25 ± 21 (62) | 0.124 ± 0.014[d] | 0.42 ± 0.06 (59) | 11 ± 1 (55) |
| Thiocytisine **2** | 27,000 ± 5000 | >100 (10)[f] | 0.832 ± 0.056 | 2.2 ± 0.5 (28) | >100 (<10) |
| Caulophylline methiodide **3** | 1500 ± 400 | >100 (29)[f] | 0.238 ± 0.046 | 41 ± 31 (70) | 37 ± 14 (60) |
| Chloro **4a** | 18 ± 10 | 5.1 ± 4.4 (71) | 0.022 ± 0.005 | 0.11 ± 0.07 (72) | 28 ± 15 (53) |
| Bromo **4b** | 7.5 ± 1.2 | 0.88 ± 0.49 (74) | 0.010 ± 0.001 | 0.050 ± 0.027 (85) | 31 ± 4 (59) |
| Iodo **4c** | 7.4 ± 1.6 | 0.55 ± 0.22 (78) | 0.017 ± 0.002 | 0.036 ± 0.024 (92) | 45 ± 15 (46) |
| Chloro **5a** | 3900 ± 700 | >100 (<10) | 2.5 ± 0.3 | 4.0 ± 0.5 (58) | >100 (<10) |
| Bromo **5b** | 380 ± 60 | 39 ± 4 | 0.308 ± 0.014 | 1.1 ± 0.6 (70) | >100 (<10) |
| Dichloro **6a** | 39,000[e] | >100 (<10) | 2.5 ± 0.04 | >100 (20)[f] | >100 (23)[f] |
| Dibromo **6b** | 45,000 ± 5000 | >100 (<10) | 10.8 ± 0.4 | >100 (23)[f] | >100 (<10) |
| Diiodo **6c** | 4200 ± 800 | >100 (<10) | 0.52 ± 0.015 | 42 ± 26 (42) | >100 (<10) |

Values represent the average ±S.E.M. of three experiments performed in triplicate.

[a] $K_i$ (nM) versus 0.5 nM $[^3H]$-(±)-epibatidine.

[b] Taken from Ref. 6.

[c] $EC_{50}$ (μM), % efficacy in parentheses relative to (±)-epibatidine.

[d] Taken from Ref. 19.

[e] Average of two experiments.

[f] Efficacy at 500 μM concentration.

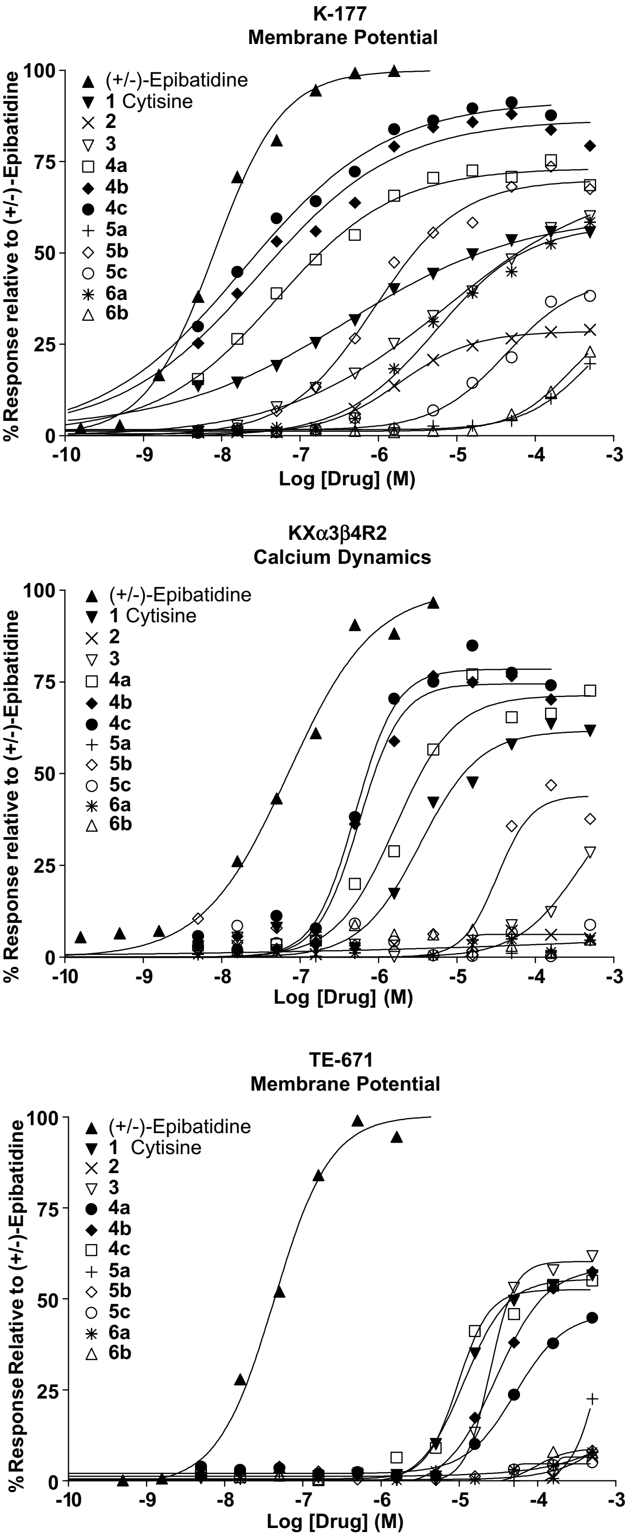

**Figure 2.** Functional data for compounds evaluated.

receptors. There is a reasonably consistent order-of-magnitude difference between the two systems, the latter exhibiting lower affinity. An exception is 5-bromocytisine, which is radically different in the two systems. This difference may reflect simple species (rat vs human) differences or differences between oocyte and brain membranes. Moreover, the binding data are derived from different radioligands [$^3$H]-epibatidine[6,19] and [$^3$H]-cytisine.[7,8] Functional data (K-177 vs oocytes) suggests differences also. For oocytes, a 10-fold increase in functional potency and no change in efficacy was seen on 3-bromination of cytisine, while 5-bromination and 3,5-dibromination produced inactive compounds. For K-177 cells, 3-bromination increased potency by around 10-fold and efficacy by more than 40% relative to cytisine. Further, the 5-bromo compound lost only around 3-fold potency and retained efficacy (70%), whereas the 3,5-dibromo compound showed minimal (23%) efficacy at the highest concentration (500 μM). Both of these compounds were inactive in oocytes. This may reflect differences in the two measurement methods (electrophysiological conductance vs fluorescence membrane potential) or cell systems (oocytes vs HEK cells).

Remarkably, methiodide **3** maintained a reasonable activity at the receptors, losing 18-fold in potency at α4β2 subtype but less than 2-fold at the neuromuscular subtype. In both cases the methiodide displayed greater efficacy than cytisine. For the α3β4 subtype, the calcium assay showed that the methiodide had only partial agonist activity, with a loss of at least 100-fold in potency. However, a single membrane potential experiment indicated that the loss in activity was only about 2-fold (data not shown) in this subtype. Binding data indicated an approximately 7-fold drop in affinity. The reason for this discrepancy is not clear.

Thiocytisine (**2**), envisioned as a bioisosteric replacement for the carbonyl function with less hydrogen-bonding activity, showed modest partial agonism at the α4β2 subtype, while being inactive at the α3β4 subtype and an extremely weak, low-efficacy partial agonist at the neuromuscular subtype.

Overall, the data suggest that halogen substitution at the 3-position is tolerated and indeed imparts greater potency, while the 5-position is not as tolerant. It is also noted that strong electron withdrawal is not tolerated by the system, possibly because of reduced hydrogen bonding ability of the carbonyl function, although untoward effects on the pi system may also be responsible.

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