# TC299423, A Novel Partial Agonist for Nicotinic Acetylcholine Receptors

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## Supplementary information

**S1 Table. Brain and Plasma concentrations of TC299423 measured by [$^{125}$I]epibatidine displacement**

**Brain measurements**

| *Time, min* | *$K_i$, pM* | *[TC299423] in assay, nM* | *[TC299423] in brain, pmol/mg[a]* |
|---|---|---|---|
| Control | 23 ± 2.3 | 0 | 0 |
| 5 | 27 ± 2.5 | 2.2 ± 1.1 | 45 ± 25 |
| 10 | 31 ± 1.7 | 4.4 ± 0.9 | 88 ± 18 |
| 20 | 29 ± 0.4 | 3.7 ± 0.2 | 73 ± 4.0 |

**Blood measurements**

| *Time, min* | *Vol serum for $IC_{50}$, µL* | *[TC299423] nM* |
|---|---|---|
| 5 | 42 ± 9.7 | 250 ± 50 |
| 10 | 28 ± 5.5 | 370 ± 70 |
| 20 | 89 ± 18 | 120 ± 30 |

Data are expressed as mean ± SEM

[a], Data are pmol TC299423 / mg of brain tissue

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**S2 Table. *In vitro* metabolism of TC299423, compared with varenicline.**

| | Human microsomes | | Rat microsomes | | Turnover by human cytochrome P450 isoforms | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Clearance[a] | $T_{1/2}$[b] | Clearance[a] | $T_{1/2}$[b] | CYP1A2[c] | CYP2C9[c] | CYP2C19[c] | CYP2D6[c] | CYP3A4[c] | Human FMO3[c] |
| Varenicline | 39 | 43 | 233 | 10 | 7 | 0 | 0 | 39 | 50 | |
| TC299423 | 5 | 285 | 18 | 79 | 3 | 8 | 4 | 42 | 7 | 17 |

The assay mixtures (final incubation volume of 125 µL) in 0.255 M phosphate buffer with 0.575% (w/v) KCl (pH 7.4) contained PiB (1.0 µM), 2.0 mM nicotinamide-adenine dinucleotide phosphate (reduced form) (NADPH), and a recombinantly expressed cytochrome P450 enzyme preparation (1A2, 2C9, 2C19, 2D6, 3A4, and FMO3) at 100 pmol/mL. After a 10 min preincubation at 37 °C, the reaction was initiated by addition of 2.0 mM NADPH and incubated for 60 min. The reactions were terminated by the addition of two volumes of DMSO. Samples were subsequently centrifuged at 4,000 rpm for 10 min. From the resulting supernatant, 10 µL was analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Control experiments were performed by substituting the active enzyme preparation by cell preparations containing no recombinant human CYP.

[a], Data for clearance are given as µL/min/mg protein.

[b], Unit for $T_{1/2}$ is min.

[c], Data for cytochrome P450 turnover and for human flavin-containing monooxygenase 3 (FMO3) are given as percent decline over 60 min.

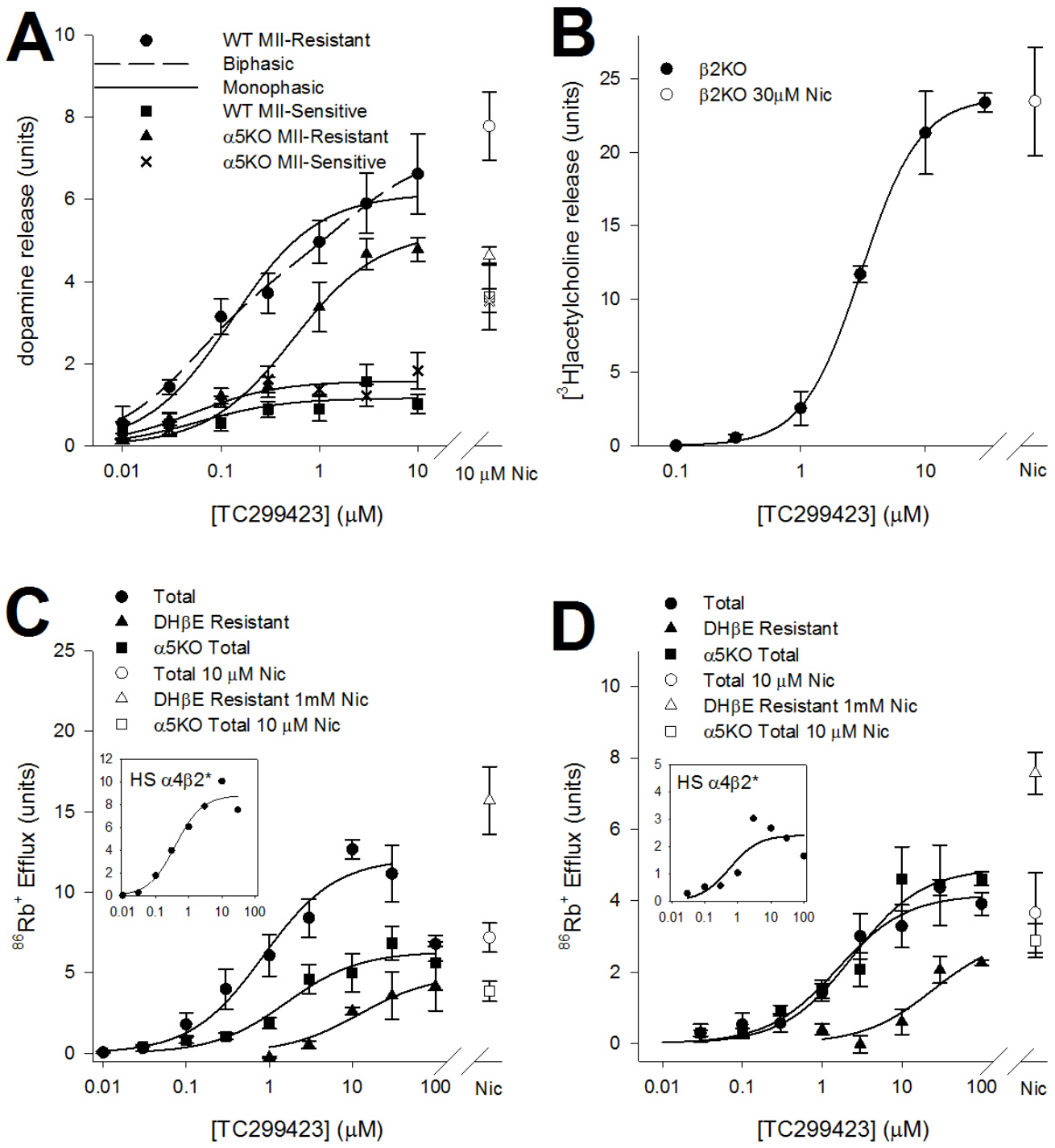
**S3 Table. Competition binding on 70 target proteins tested at a concentration of 1 μM TC299423**

| Inhibition | Assay Name | Radioligand/Substrate | Hit |
|---|---|---|---|
| **12.86%** | Adenosine Transporter (h) | [3H]-NBTI | FALSE |
| **7.85%** | Adenosine, A1 | [3H]CPX | FALSE |
| **1.51%** | Adenosine, A2A (h) | [3H]CGS 21680 | FALSE |
| **-0.05%** | Adrenergic, Alpha 1A | [3H]-7-MeOxy-Prazosin | FALSE |
| **-8.31%** | Adrenergic, Alpha 1B | [3H]-7-MeOxy-Prazosin | FALSE |
| **6.75%** | Adrenergic, Alpha 2A (h) | [3H]MK-912 | FALSE |
| **-14.09%** | Adrenergic, Alpha 2B | [3H]MK-912 | FALSE |
| **6.28%** | Adrenergic, Alpha 2C (h) | [3H]MK-912 | FALSE |
| **11.21%** | Adrenergic, Beta 1 (h) | [125I] (-) Iodocyanopindolol | FALSE |
| **-8.25%** | Adrenergic, Beta 2 (h) | [125]I-Iodo-cyanopindolol | FALSE |
| **-5.87%** | Dopamine Transporter | [3H]WIN 35,428 | FALSE |
| **-2.94%** | Dopamine, D1 (h) | [3H]-SCH23390 | FALSE |
| **-21.17%** | Dopamine, D2s (h) | [3H]-Raclopride | FALSE |
| **-16.93%** | Dopamine, D3 | [3H]7-OH-DPAT | FALSE |
| **-2.44%** | Dopamine, D4.4 (h) | [3H]-YM-09151-2 | FALSE |
| **-2.66%** | GABA A, Agonist Site | [3H]GABA | FALSE |
| **11.84%** | GABA A, BDZ, alpha 1 site | [3H]Flunitrazepam | FALSE |
| **-2.43%** | GABA-B | [3H]CGP 54626A | FALSE |
| **-1.26%** | Glutamate, AMPA Site (Ionotropic) | [3H]AMPA | FALSE |
| **-6.93%** | Glutamate, Kainate Site (Ionotropic) | [3H]Kainic acid | FALSE |
| **5.46%** | Glutamate, MK-801 Site (Ionotropic) | [3H]MK-801 | FALSE |
| **5.51%** | Glutamate, NMDA Agonist Site (Ionotropic) | [3H]CGP 39653 | FALSE |
| **7.71%** | Glutamate, NMDA, Phencyclidine Site (Ionotropic) | [3H]TCP | FALSE |
| **4.34%** | Glutamate,NMDA,Glycine (Stry-insens Site) (Ionot | [3H]-MDL-105,519 | FALSE |
| **-7.63%** | Glycine, Strychnine-sensitive | [3H]Strychnine | FALSE |
| **15.56%** | Histamine, H1 | [3H]Pyrilamine | FALSE |
| **6.50%** | Histamine, H2 | [125I]-Aminopotentidine | FALSE |

| | | | |
|---|---|---|---|
| **5.85%** | Histamine, H3 | [3H]N-a-MeHistamine | FALSE |
| **-11.73%** | Muscarinic, M1 (hr) | [3H]Scopolamine, N-Methyl | FALSE |
| **-3.18%** | Muscarinic, M2 (h) | [3H]Scopolamine, N-Methyl | FALSE |
| **-4.77%** | Muscarinic, M3 (h) | [3H]Scopolamine, N-Methyl | FALSE |
| **-6.69%** | Muscarinic, M4 (h) | [3H]Scopolamine, N-Methyl | FALSE |
| **2.24%** | Muscarinic, M5 (h) | [3H]Scopolamine, N-Methyl | FALSE |
| **93.33%** | **Nicotinic, Neuronal (α-BnTx insensitive)** | **[3H]Epibatidine** | **TRUE** |
| **23.72%** | Norepinephrine Transporter | [3H]Nisoxetine | FALSE |
| **-2.12%** | Opioid, Delta 2 (h) | [3H]-Naltrindole | FALSE |
| **-6.43%** | Opioid, Mu (h) | [3H]-Diprenorphine | FALSE |
| **7.03%** | Serotonin Transporter | [3H]Citalopram, N-Methyl | FALSE |
| **17.60%** | Serotonin, 5HT1A (h) | [3H]-8-OH-DPAT | FALSE |
| **9.99%** | Serotonin, 5HT1D | [3H]5-CT | FALSE |
| **12.43%** | Serotonin, 5HT2A | [3H]Ketanserin | FALSE |
| **-2.21%** | Serotonin, 5HT2C | [3H]Mesulergine | FALSE |
| **7.50%** | Serotonin, 5HT3 | [3H]GR 65630 | FALSE |
| **-16.64%** | Serotonin, 5HT4 | [3H]GR 113808 | FALSE |
| **1.24%** | Serotonin, 5HT5A (h) | [3H]-LSD | FALSE |
| **8.87%** | Serotonin, 5HT6 (h) | [3H]-LSD | FALSE |
| **-2.10%** | Serotonin, 5HT7 (h) | [3H]LSD | FALSE |
| **-0.52%** | Sigma 1 | [3H]-(+)-Pentazocine | FALSE |
| **-0.65%** | Sigma 2 | [3H]-DTG | FALSE |
| **-3.91%** | Calcium Channel, Type L (Dihydropyridine Site) | [3H]Nitrendipine | FALSE |
| **-0.60%** | Calcium Channel, Type N | [125I]-Conotoxin GVIA | FALSE |
| **6.83%** | GABA, Chloride, TBOB Site | [3H]TBOB | FALSE |
| **52.12%** | **Potassium Channel, ATP-Sensitive** | **[3H]Glibenclamide** | **TRUE** |
| **-2.57%** | Potassium Channel, Ca2+ Act., VI | [125I]Apamin | FALSE |
| **14.46%** | Potassium Channel, I[Kr] (hERG) (h) | [3H]Astemizole | FALSE |
| **6.91%** | Sodium, Site 2 | [3H]Batrachotoxin A 20-a-Benzo | FALSE |

| | | | |
|---|---|---|---|
| **-10.86%** | Nitric Oxide, NOS (Neuronal-Binding) | [3H]NOARG | FALSE |
| **29.86%** | Leukotriene, LTB4 (BLT) | [3H]LTB4 | FALSE |
| **-8.26%** | Leukotriene, LTD4 (CysLT1) | [3H]LTD4 | FALSE |
| **3.14%** | Thromboxane A2 (h) [SQ 29,548] | 3H SQ 29,548 | FALSE |
| **24.85%** | Angiotensin II, AT1 (h) | [125I]-(Sar1-Ile8) Angiotensin | FALSE |
| **32.29%** | Bradykinin, BK2 | [3H]Bradykinin | FALSE |
| **8.93%** | Endothelin, ET-A (h) | [125I] Endothelin-1 | FALSE |
| **2.10%** | Neurokinin, NK1 | [3H]Substance P | FALSE |
| **2.16%** | Neuropeptide, NPY2 (h) | [125I]-PYY | FALSE |
| **21.55%** | Esterase, Acetylcholine | Acetylthiocholine | FALSE |
| **e12.00%** | Phosphodiesterase, PDE4A1A (h) | Fluorescent cyclic AMP | FALSE |
| **-3.00%** | Phosphodiesterase, PDE5A1 (h) | Fluorescent cyclic GMP | FALSE |
| **-5.00%** | Kinase, Protein, PKA (h) | Fluorescein-labeled peptide | FALSE |
| **-1.00%** | Kinase, Protein, PKCa (h) | Fluorescein-labeled peptide | FALSE |

TC299423 inhibited >50% of the reference radioligand for two sites: Neuronal nAChRs and ATP-sensitive potassium channels. 1µM TC299423 inhibited 93.33% of the [$^3$H]-epibatidine binding at nAChRs and 52.12% of the [$^3$H]-Glibenclamide binding at the ATP-sensitive potassium channels.




**S1 Figure. Functional assays for measuring TC299423 agonist activity at various nAChRs.** All points are mean ± SEM. **A)** Closed symbols show α-CtxMII-resistant [$^3$H]-dopamine release and α-CtxMII-sensitive [$^3$H]-dopamine release from WT and α5KO striatal synaptosomes. Open symbols show [$^3$H]-dopamine release evoked by 10 μM nicotine for the matching closed symbols. **B)** Measurement of functional α3β4*-nAChR by [$^3$H]-ACh release from β2KO IPN synaptosomes. Curve fit $EC_{50}$ and efficacy values for (**A)** and (**B)** are shown in Table 1 (see Results). **C)** Activity of TC299423 on α4β2* measured by high-sensitivity $^{86}Rb^+$ efflux from WT

and α5KO thalamic synaptosomes. 2 μM DHβE was used to isolate the high-sensitivity and low-sensitivity responses. **D)** Activity of TC299423 on α4β2* measured by high-sensitivity $^{86}Rb^{+}$ efflux from WT and α5KO cortical synaptosomes. As in C, 2 μM DHβE was used to distinguish between high-sensitivity and low-sensitivity nAChRs. Calculated $EC_{50}$ and efficacy values for **(C)** and **(D)** are presented in Table 2 (see Results).