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Supporting Information

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Title:

Chemical Medicine: Novel 10-Substituted Cytisine Derivatives with Increased Selectivity for a4ß2 Nicotinic Acetylcholine Receptors

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Contents:

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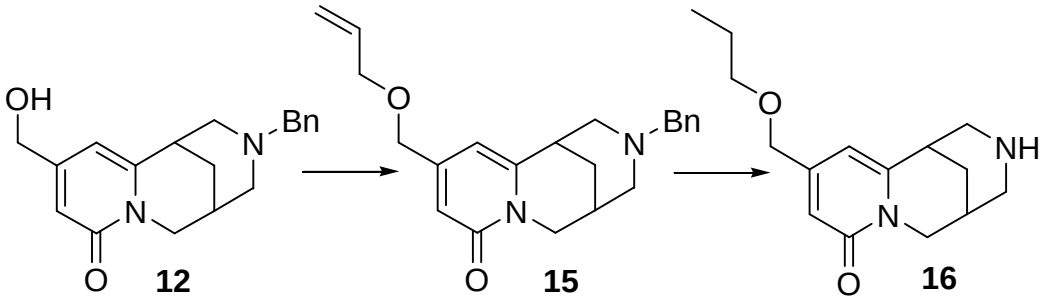
[**] *Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.*

I. Experimental Section:

General Chemistry Methods: All solvents and reagents were used as obtained from commercial sources unless otherwise indicated. All starting materials were also obtained from commercial source. All reactions were performed under argon unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on an Avance 400 Bruker instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Deuterated chloroform (99.8%D) or methanol (99.8%D) was used as solvents. ^{1}H Chemical shifts value (*d*), from tetramethylsilane as internal standard. ^{13}C chemical shifts (*d*) are referenced to $CDCl_3$ (central peak, *d* = 77.00ppm) and CD_3OD (central peak, *d* = 49.15ppm) as the internal standard. Mass spectra were measured in positive mode electrospray ionization (ESI). The HRMS data were obtained on a Micromass Q-TOF-2TM instrument. TLC was performed on silica gel 60 F_{254} glass plates; column chromatography was performed using silica gel (35-75 mesh). All final compounds send for biological assay are further purified by HPLC. Analytical HPLC was performed using a Shimadzu LC-10AD system, equipped with a Waters 484 tunable absorbance detector set at 254, or 280nm. The HPLC data of tested compounds including the conditions are given in Table 1.

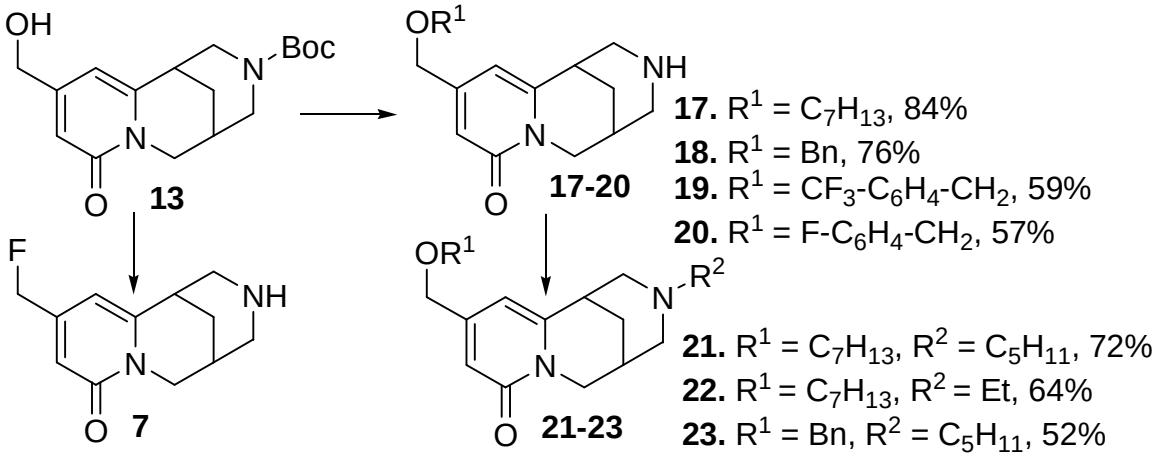
10-CH_2OR derivatives of Cytisine



10-(Allyloxymethyl)-3-benzyl--1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-15): To a stirred solution of the alcohol *rac*-**12**, (20 mg, 0.06 mmol) in dry THF (1 ml) at 0 °C under argon was added NaH (55% by wt., 3 mg, 0.07 mmol). After 30 min, catalytic amount (2.4 mg) of *tert*-butyl ammonium iodide (TBAI) and allyl bromide (0.01 ml, 0.13 mmol) was added and allowed the reaction to warm to room temperature. The reaction was completed in 3.5 h. After cooling, quenched the reaction mixture with saturated ammonium chloride solution and extracted the organic layer with ethyl acetate. The crude product was purified using a semi-preparative HPLC to get 22 mg (97%) of the allyl ether derivative ***rac*-15**.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45-7.38 (m, 3H), 7.32 (d, 2H, *J* = 6.6 Hz), 6.50 (s, 1H), 6.32 (s, 1H), 5.94-5.86 (m, 1H), 5.32-5.22 (m, 2H0, 4.38-4.15 (m, 5H), 4.03-4.01 (m, 3H), 3.70-3.65 (m, 2H), 3.33 (s, 1H), 3.10 (d, 2H, *J* = 10.6 Hz), 2.83 (s, 1H), 1.99 (br s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.6, 145.5, 133.5, 131.2, 129.7, 128.8, 127.6, 117.4, 113.4, 107.4, 71.4, 68.9, 60.9, 55.9, 55.5, 48.2, 33.0, 26.1, 23.5.

10-(Propoxymethyl)-3-benzyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-16): To a mixture of the above N-Benzyl allyl ether cytisine derivative rac-**15**, (21 mg, 0.06 mmol) and Boc-anhydride (26 mg, 0.12 mmol) in degassed MeOH was added 4.5 mg of 20% $Pd(OH)_2$-C, degassed three times and refluxed the reaction mixture under 1 atm H_2 pressure for 5 min. Cooled the reaction mixture and the catalyst was removed by filtration, washed with MeOH and concentrated. After the usual aqueous work up with EtOAc and evaporation of the solvent, the crude product was purified using semi preparative HPLC (CH_3CN/H_2O mixture in 0.05% TFA) to the N-Boc protected 10-propoxy methyl derivative. The above N-Boc protected 10-propoxymethyl derivative (6 mg, 0.02 mmol) in a mixture of TFA/DCM (0.02 ml/0.2 ml) was stirred at room temperature for 45 min. After removing the excess TFA, the crude mass was purified using preparative HPLC (CH_3CN/H_2O mixture in 0.05 % TFA). Concentrated and dried under vacuo to afford 3 mg (69 %) of the pure 10-propoxymethyl cytisine *rac*-**16**. ^{1}H NMR (MeOD, 400 MHz): δ 6.44 (s, 1H), 6.31 (s, 1H), 4.36 (s, 2H), 4.09 (d, 1H, *J* = 15.9 Hz), 3.91 (dd, *J* = 9.2, 6.6 Hz), 3.43-3.27 (m, 8H), 2.69 (br s, 1H), 2.05 (dd, 2H, *J* = 14.5, 13.5 Hz), 1.58 (q, 2H, *J* = 7.0 Hz), 0.90 (t, 3H, *J* = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.7, 150.7, 150.6, 113.5, 103.8, 72.6, 70.9, 53.9, 52.9, 49.6, 35.7, 27.7, 26.3, 22.8, 10.6. MS (ESI) 263.17 $[M+H]^+$; HRMS (ESI) calculated for $C_{15}H_{23}N_2O_2^+$ $[M+H]^+$ 263.1767; found, 263.1760.



Representative experimental procedure for O-alkylation, N-Boc deprotection and N-alkylation of cytisine (*rac*-17 and *rac*-18):

10-Cyclohexyl(methoxymethyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-17): To a stirred solution of the alcohol *rac*-**13**, (20 mg, 0.06 mmol) in dry THF (1 ml) at 0 °C under argon was added NaH (60% by wt., 7.5 mg, 0.19 mmol). After stirring the mixture for 30 min, catalytic amount (1.2 mg, 0.003 mmol) of *tert*-butyl ammonium iodide (TBAI) and bromomethyl-cyclohexane (0.02 ml, 0.14 mmol) was added and allowed the reaction mixture to warm to room temperature and stirred overnight. After cooling, quenched the reaction mixture with saturated ammonium chloride solution and extracted the organic layer with ethyl acetate, dried and concentrated. The crude product was

purified using a semi-preparative HPLC to get 12 mg (46%) of the boc protected derivative which was further treated with a mixture of TFA/DCM (0.07ml:1ml) at ice temperature and slowly warmed to room temperature during 3 h. After removing the excess TFA, the crude product was purified using semipreparative HPLC (CH_3CN/H_2O mixture in 0.05 % TFA), to afford 7 mg (84 %) of the pure 10-cyclohexyl(methoxymethyl) cytisine *rac*-**17**.

^{1}H NMR (MeOD, 400 MHz): δ 6.51 (s, 1H), 6.28 (s, 1H), 4.29(s, 2H), 4.08 (d, 1H, *J* = 5.8 Hz), 3.90 (dd, 1H, *J* = 9.2, 6.7 Hz), 3.40-3.24 (m, 6H), 2.68 (br s, 1H), 2.04 (dd, 2H, *J* = 14.8, 13.4 Hz), 1.72-1.52 (m, 6H), 1.22-1.10 (m, 5H), 0.96-0.87 (m, 2H). ^{13}C NMR (MeOD, 100 MHz): δ 165.9, 154.5, 147.8, 115.1, 107.8, 78.2, 71.7, 51.0, 49.9, 49.7, 39.6, 33.4, 31.3, 27.8, 27.1, 26.8, 24.5. MS (ESI) 317.3 $[M+H]^+$; HRMS (ESI) calculated for $C_{19}H_{29}N_2O_2^+$ $[M+H]^+$ 317.2229; found, 317.2224.

10-(benzyloxymethyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-18): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37-7.31 (m, 5H), 6.45 (s, 1H), 6.07 (s, 1H), 4.57 (s, 2H), 4.38 (d, 2H, *J* = 1.9 Hz), 4.13 (d, 1H, *J* = 15.5 Hz), 3.89 (dd, 1H, *J* = 9.0, 6.6 Hz), 3.16-3.02 (m, 4H), 2.93 (s, 1H), 2.36 (br s, 1H), 1.97 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.4, 150.3, 137.7, 128.5, 127.8, 127.7, 113.8, 103.9, 72.6, 70.2, 53.6, 52.7, 49.2, 35.5, 27.6, 26.2. MS (ESI) 311.1 $[M+H]^+$; HRMS (ESI) calculated for $C_{19}H_{23}N_2O_2^+$ $[M+H]^+$ 311.1760; found, 311.1767.

10-(4-Trifluoromethyl-benzyloxymethyl) -1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-19): (^{1}H NMR, 400 MHz, D_2O): δ 7.54 (d, 2H, *J* = 8.1 Hz), 7.39 (d, 2H, *J* = 8.0 Hz), 6.36 (d, 2H, *J* = 9.2 Hz), 4.54 (s, 2H), 4.37 (s, 2H), 3.98 (d, 1H, *J* = 15.7 Hz), 3.82 (dd, 1H, *J* = 9.0, 6.6 Hz), 3.36-3.20 (m, 5H), 2.66 (br s, 1H), 1.97 (d, 1H, *J* = 13.7 Hz), 1.89 (d, 1H, *J* = 13.8 Hz). (^{13}C NMR, 100 MHz, D_2O): δ 164.8, 152.4, 146.9, 141.1, 128.7, 125.4 (d, *J* = 3.9 Hz), 114.3, 108.6, 72.1, 69.9, 49.5, 48.6, 48.2, 31.5, 24.8, 22.6. MS (ESI) 379.1 $[M+H]^+$; HRMS (ESI) calculated for $C_{20}H_{22}F_3N_2O_2^+$ $[M+H]^+$ 379.1633; found, 379.1632.

10-(4-Fluoro-benzyloxymethyl) -1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-20): (^{1}H NMR, 400 MHz, D_2O): δ 7.26 (dd, 2H, *J* = 5.6, 2.9 Hz), 6.98 (t, 2H, *J* = 8.9 Hz), 6.39 (s, 1H), 6.36 (s, 1H), 4.45 (s, 2H), 4.37 (s, 1H), 4.00 (d, 1H, *J* = 15.7 Hz), 3.84 (dd, 1H, *J* = 15.7 Hz), 3.84 (dd, 1H, *J* = 9.1, 6.5 Hz), 3.38-3.21 (m, 5H), 2.68 (br s, 1H), 1.95 (dd, 2H, *J* = 16.5, 13.7 Hz). (^{13}C NMR, 100 MHz, D_2O): δ 164.8, 163.7, 152.6, 146.9, 132.8, 130.7 (d, *J* = 8.4 Hz), 115.3 (d, *J* = 21.5), 114.2, 108.7, 72.1, 69.6, 49.5, 48.6, 48.2, 31.5, 24.8, 22.6. MS (ESI) 329.1 $[M+H]^+$; HRMS (ESI) calculated for $C_{19}H_{22}F_1N_2O_2^+$ $[M+H]^+$ 329.1665; found, 329.1665.

10-Cyclohexyl(methoxymethyl)-3-pentyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-21): A mixture of 10-cyclohexyl(methoxymethyl) cytisine **17** (9 mg, 0.03 mmol) and n-pentane bromide (0.002 ml, 0.02 mmol) in dry acetone (1.5 ml) under argon was refluxed

overnight. After cooling and removing the solvent under vacuum, the crude mixture was diluted with ethyl acetate. Usual aqueous work up and purification by semi preparative HPLC afforded 8 mg (72%) of the desired product *rac*-**21**.

^{1}H NMR (MeOD, 400 MHz): δ 6.51 (s, 1H), 6.37 (d, 1H, *J* = 1.4 Hz), 4.37 (s, 1H), 4.12 (d, 1H, *J* = 15.9 Hz), 3.99 (dd, 1H, *J* = 9.0, 6.8 Hz), 3.67 (d, 1H, *J* = 13.2 Hz), 3.59 (d, 1H, *J* = 12.4 Hz), 3.46 (br s, 1H), 3.37 (br s, 1H), 3.06 (t, 2H, *J* = 8.5 Hz), 2.83 (br s, 1H), 2.09 (d, 2H, *J* = 2.8 Hz), 1.80-1.63 (m, 9H), 1.38-1.24 (m, 9H), 1.05-0.9 (m, 2H), 0.92 (t, 3H, *J* = 7.0 Hz)). ^{13}C NMR (MeOD, 100 MHz): δ 165.7, 154.4, 147.4, 115.1, 107.6, 78.1, 71.6, 59.8, 59.1, 58.4, 39.5, 34.3, 31.1, 29.7, 27.9, 27.7, 26.9, 24.3, 24.2, 23.1, 14.1. HRMS (ESI) calculated for $C_{24}H_{39}N_2O_2^+$ $[M+H]^+$ 387.3012; found, 387.3008.

10-Cyclohexyl(methoxymethyl)-3-ethyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-22): ^{1}H NMR (MeOD, 400 MHz): δ 6.53 (s, 1H), 6.38 (d, 1H, *J* = 1.5 Hz), 4.38 (s, 2H), 4.13 (d, 1H, *J* = 15.9 Hz), 4.01 (dd, 1H, *J* = 9.2, 6.6 Hz), 3.69 (d, 1H, *J* = 13.1 Hz), 3.60 (d, 1H, *J* = 12.4 Hz), 3.48-3.47 (m, 1H), 3.35-3.31 (m, 2H), 3.18 (q, 2H, *J* = 7.0 Hz), 2.85 (br s, 1H, 2.11-2.10 (m, 2H), 1.82-1.76 (m, 5H), 1.29-1.22 (m, 9H), 1.06-0.99 (m, 2H). ^{13}C NMR (MeOD, 100 MHz): δ 163.9, 152.6, 145.6, 113.3, 105.8, 76.3, 69.8, 56.8, 56.0, 53.3, 37.6, 32.4, 29.3, 26.1, 25.9, 25.2, 22.4, 7.4. MS (ESI) 345.2 $[M+H]^+$; HRMS (ESI) calculated for $C_{21}H_{33}N_2O_2^+$ $[M+H]^+$ 345.2542; found, 345.2535.

10-(Benzyloxymethyl)-3-pentyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-23): ^{1}H NMR (MeOD, 400 MHz): δ .7.39-7.29 (m, 5H), 6.56 (s, 1H), 6.41 (d, 1H, *J* = 1.3 Hz), 4.62 (s, 2H), 4.46 (s, 2H), 4.00 (dd, 1H, *J* = 8.8, 6.6 Hz), 3.67 (d, 1H, *J* = 12.1 Hz), 3.59 (d, 1H, *J* = 12.6), 3.47 (s, 1H), 3.07 (t, 2H, *J* = 8.5 Hz), 2.84 (br s, 1H), 1.66-1.63 (m, 2 H), 1.38-1.29 (m, 6H), 0.92 (t, 3H, *J* = 7.0 Hz). ^{13}C NMR (MeOD, 100 MHz): δ 165.7, 154.0, 148.9, 147.6, 129.7, 129.2, 115.5, 107.9, 74.2, 70.9, 59.9, 59.2, 58.5, 34.4, 29.8, 28.1, 24.5, 24.4, 23.3, 14.2. MS (ESI) 381.2 $[M+H]^+$; HRMS (ESI) calculated for $C_{24}H_{33}N_2O_2^+$ $[M+H]^+$ 381.2542; found, 381.2538.

10-Fluoromethyl -1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-*a*][1,5]diazocin-8-one (*rac*-7): To a stirred solution of *rac*-**13** (26 mg, 0.081 mmol) in CH_2Cl_2 (1 ml) at -78 °C under argon was added dropwise diethylaminosulphurtrifluoride, DAST (0.021 ml, 0.16 mmol). The reaction mixture was allowed to warmed to room temperature in 3 h. Normal aqueous work up followed by purification of the crude product by semi preparative HPLC gave 9 mg of the Boc protected cytisine intermediate.

(^{1}H NMR, 400 MHz, $CDCl_3$): δ 6.41 (s, 1H), 6.06 (s, 1H), 5.29 (s, 1H), 5.17 (s, 1H), 4.24-4.16 (m, 3H), 3.84 (dd, 1H, *J* = 9.1, 6.4 Hz), 3.10-3.02 (m, 3H), 2.45 (s, 1H), 1.99-1.97 (m, 2H), 1.35-1.19 (m, 9H). After Boc deprotection using TFA/DCM mixture and purification by semipreparative HPLC, *rac*-**7** was obtained in quantitative yield.

(^{1}H NMR, 400 MHz, D_2O): δ 6.49 (d, 2H, *J* = 12.9 Hz), 5.37 (s, 1H), 5.26 (s, 1H), 4.10 (d, 1H, *J* = 15.7 Hz), 3.96 (dd, 1H, *J* = 9.1, 6.6 Hz), 3.47-3.41 (m, 2H), 3.37-3.3(m, 3H), 2.77 (br s, 1H), 2.10-1.99 (m, 2

H). (^{13}C NMR, 100 MHz, D_2O): δ 164.8, 151.2, 147.3, 112.6, 106.7, 82.9, 81.2, 49.5, 48.7, 48.3, 31.6, 24.8, 22.6.

MS (ESI) 223.12 $[M+H]^+$; HRMS (ESI) calculated for $C_{12}H_{16}F_1N_2O_1^+$ $[M+H]^+$ 223.1247; found, 223.1238.

II. Table 1: HPLC Purity Analysis Data Sheet

Compd No.	Solvent system	Wavelength	t_R (min)	Purity	HPLC column
rac-7	B	280	8.6	97%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-7	C	280	13.7	98%	Inertsil 10u C8 (250 X4.6mm)
rac-10	A	280	15.7	97%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-12	A	280	8.7	97%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-12	C	280	15.0	97%	Inertsil 10u C8 (250 X4.6mm)
rac-13	A	280	11.5	99%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-13	C	280	13.3	98%	Inertsil 10u C8 (250 X4.6mm)
rac-14	B	280	18.9	98%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-14	C	280	15.4	98%	Inertsil 10u C8 (250 X4.6mm)
rac-15	B	280	17.2	97%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-15	C	280	13.2	97%	Inertsil 10u C8 (250 X4.6mm)
rac-16	A	280	11.2	97%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-16	C	280	17.5	96%	Inertsil 10u C8 (250 X4.6mm)
rac-17	A	280	12.8	96%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-17	C	280	15.1	96%	Inertsil 10u C8 (250 X4.6mm)
rac-18	A	280	13.3	97%	ACE 3 AQ (100 mm X 4.6 mm; 3.5 μm)
rac-18	C	280	16.3	97%	Inertsil 10u C8 (250 X4.6mm)

Condition **A**: 2.0 mL/min.; Gradient from 10% acetonitrile in water (0.05% TFA) to 100% acetonitrile (0.05% TFA) in 20 min.

Condition **B**: 2.0 mL/min.; Gradient from water (0.05% TFA) to 50% acetonitrile (0.05% TFA) in 20 min.

Condition **C**: 1.3 mL/min.; Gradient from 10% acetonitrile in water (0.05% TFA) to 100% acetonitrile (0.05% TFA) in 30 min.