Supporting Information

Tacripyrines, the First Tacrine-Dihydropyridine Hybrids, as Multi-Target-Directed Ligands for the Treatment of Alzheimer´s Disease

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General Methods. Reactions were monitored by tlc (thin layer chromatography) using precoated silica gel aluminium plates containing a fluorescent indicator. Detection was done by UV (254 nm) followed by charring with sulfuric-acetic acid spray, 1% aqueous potassium permanganate solution or 0.5% phosphomolybdic acid in 95% EtOH. Anhydrous Na_2SO_4 was used to dry organic solutions during work-ups and the removal of solvents was carried out under vacuum with a rotary evaporator. Flash column chromatography was performed using silica gel 60 (230-400 mesh). Melting points are uncorrected. ^{1}H and and ^{13}C NMR spectra were recorded using tetramethylsilane as internal standard. All the assignments for protons and carbons were in agreement with 2D COSY, gHSQC, gHMBC, and 1D NOESY spectra. Values with (*) can be interchanged. Compounds **15**,[30] **16**,[31] **17**,[32] **18**,[33] **19**,[34] **20**,[35] **21**, **23-25**,[36] **26**,[37] **27**[38] and **28**[38] have been synthesized as described, and isolated as a mixtures of *Z* and *E* isomers, that we have not tried to separate, but were submitted together to further reaction.

Ethyl ester of 2-(4'-biphenylmethylene)-3-oxobutanoic acid (22). To a solution of ethyl acetoacetate (1.42 g, 10.9 mmol) in dry toluene (30 mL), biphenyl-4-carboxaldehyde (2.0 g, 10.9 mmol) and piperidine (15 drops) were added. After 4 h at rt, the solvent was evaporated and the crude was purified by chromatography, affording compound **22** (2.45 g, 76%, as a mixture of *Z*/*E* isomers in a 67:33 ratio): oil; IR (KBr) ν 3072, 2985, 1716, 1662, 1623, 1603, 1487, 1232 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz) (major *Z* isomer) δ 7.75-7.38 (m, 10 H, C_6H_4-C_6H_5, HC=C), 4.35 (q, *J*= 6.9 Hz, 2 H, O*C*H_2CH$_3$), 2.45 [s, 3 H, CH_3(CO)], 1.56 (t, *J*= 6.9 Hz, 3 H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) (major *Z* isomer) δ 203.3 (C=O), 194.4 (CO_2), 140.6 (*C*H=C), 134.0 (CH=*C*), 131.6, 130.2, 130.0, 128.8, 128.7, 128.1, 127.9, 127.4, 126.8 (aromatics), 61.8 (O*C*H$_2$CH3), 26.5 [*C*H$_3$(CO)], 13.8 (*C*H$_3$CH$_2$O); MS (API-ES+) *m*/*z*:

$[M+1]^+$ 295.2; $[M+Na]^+$ 317.0; $[2M+Na]^+$, 611.2. Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.32; H, 5.97.

General Method for the synthesis of ethyl esters of (±)-6-amino-4-aryl-5-cyano-2-methyl-1,4-dihydropyridine-3-carboxylic acids (31-44). A solution of ethyl cyanoacetimidate hydrochloride (**30**)[40] (1 equiv) and ammonium acetate (1-5 equiv) in methanol was refluxed for 15 min. Then, the appropriate ethyl ester of 2-(arylmethylene)-3-oxobutanoic acid (**15-28**) (1 equiv) was added, and the mixture was refluxed for 15 min. Once the reaction was complete (tlc analysis), the reaction was cooled at 5 ºC overnight giving a crystalline precipitate that was separated and recrystallized from methanol.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylic acid (31). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (321.9 mg, 0.91 mmol) and ammonium acetate (541.0 mg, 7.02 mmol), in methanol (15 mL) with compound **15** (400 mg, 1.8 mmol), in 30 min, gave product **31** (410 mg, 80%), which showed identical data to those described in the literature.[41]

Ehyl ester of 6-amino-5-cyano-4-(4'-fluorophenyl)-2-methyl-1,4-dihydropyridine-3-carboxylic acid (32). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (820.9 mg, 5.51 mmol) and ammonium acetate (1.27 g, 16.53 mmol), in methanol (15 mL), with compound **16** (1.0 g, 4.24 mmol), in 30 min, provided product **32** (987 mg, 78%): mp 223-225 ºC; IR (KBr) ν 3420, 3354, 3231, 2982, 2869, 2181, 1666, 1496, 1369, 1329, 1270, 1222 cm^{-1}; ^1H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table8, **Supporting Information**); MS

(API-ES+) *m/z*: $[M+1]^+$ 302.1; $[M+Na]^+$ 324.0; $[2M+1]^+$ 603.3; $[2M+Na]^+$ 625.3. Anal. Calcd. for $C_{16}H_{16}N_3O_2F$: C, 63.78; H, 5.35 N, 13.95. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-(2'-trifluoromethylphenyl)-1,4-dihydropyridine-3-carboxylic acid (33). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (437.4 mg, 2.93 mmol) and ammonium acetate (733.49 mg, 9.51 mmol), in methanol (15 mL), with compound **17** (700.0 mg, 2.44 mmol), after 30 min, afforded product **33** (368.0 mg, 43%): mp 243-245 ºC; IR (KBr) ν 3401, 3340, 3224, 2992, 2833, 2182, 1657, 1624, 1489, 1367, 1255 cm^{-1}; ^1H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m/z*: $[M+1]^+$ 352.3; $[M+Na]^+$ 374.2; $[2M+Na]^+$ 723.5. Anal. Calcd. for $C_{17}H_{16}F_3N_3O_2$: C, 58.12; H, 4.59 N, 11.96. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-(2'-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (34). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (747.63 mg, 5.06 mmol) and ammonium acetate (1.2 g, 16.30 mmol), in methanol (15 mL), with compound **18** (1.1 g, 4.18 mmol), in 40 min, furnished product **34** (511 mg, 38%): mp 123-125 ºC; IR (KBr) ν 3382, 3210, 2978, 2218, 1715, 1609, 1557, 1348, 1276 cm^{-1}; ^1H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m/z*: $[M+1]^+$ 329.1; $[M+Na]^+$ 351.1; $[2M+1]^+$ 657.3; $[2M+Na]^+$ 679.2. Anal. Calcd. for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91: N, 17.06. Found: C, H, N.

Ehyl ester of 6-amino-5-cyano-2-methyl-4-(3'-nitrophenyl)-1,4-dihydropyridine-carboxylic acid (35). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (679.6 mg, 3.57 mmol) and ammonium acetate (1.14 g, 12.48 mmol), in methanol (15 mL), with compound **19** (1.0 g, 3.2 mmol), after 20

min, gave product **35** (950 mg, 90%): mp 218-220 ºC; IR (KBr) ν 3402, 3362, 3230, 2184, 1658, 1530, 1486, 1348, 1274 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 329.1; $[M+Na]^+$ 351.1; $[2M+1]^+$ 657.3; $[2M+Na]^+$ 679.2. Anal. Calcd. for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91: N, 17.06. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-(4'-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (36). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (747.6 mg, 5.06 mmol) and ammonium acetate (1.25 g, 16.30 mmol), in methanol (15 mL), with compound **20** (1.1 g, 4.18 mmol), after 30 min, provided product **36** (796 mg, 67%): mp 240-242 ºC; IR (KBr) ν 3407, 3346, 3230, 2898, 2177, 1665, 1605, 1518, 1494, 1348, 1274 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 329.0; $[M+Na]^+$ 351.0; $[2M+1]^+$ 657.3; $[2M+Na]^+$ 679.2. Anal. Calcd. for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.06. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-(4'-methylphenyl)-1,4-dihydropyridine-3-carboxylic acid (37). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (769,79 mg, 5,16 mmol) and ammonium acetate (1.29 g, 16.77 mmol), in methanol (15 mL), with compound **21** (1.0 g, 4.3 mmol), after 35 min, gave product **37** (822.0 mg, 65%): mp 220-222 ºC; IR (KBr) ν 3407, 3347, 3227, 2181, 1664, 1405, 1321, 1222 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 298.1; $[M+Na]^+$ 320.1; $[2M+1]^+$ 595.2; $[2M+Na]^+$ 617.3. Anal. Calcd. for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, H, N.

Ethyl ester of 6-amino-4-(4'-biphenyl)-5-cyano-2-methyl-1,4-dihydroyridine-3-carboxylic acid (38). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (303.89 mg, 2.03 mmol) and ammonium acetate (507.83 mg, 6.59 mmol), in methanol (15 mL), with compound **22** (500 mg, 1.69 mmol), after 35 min, provided compound **38** (363 mg, 60%): mp 217-219 ºC; IR (KBr) ν 3405, 3346, 3225, 2876, 2179, 1630, 1662, 1494, 1369, 1270, 1221 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m/z*: $[M+1]^+$ 360.1; $[M+Na]^+$ 382.1; $[2M+1]^+$ 719.2; $[2M+Na]^+$ 741.2. Anal. Calcd. for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-4-(2'-methoxyphenyl)-2-methyl-1,4-dihydropiridine-3-carboxylic acid (39). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (720.18 mg, 4.83 mmol) and ammonium acetate (1.20 g, 15.67 mmol), in methanol (15 mL), with compound **23** (1.0 g, 4.02 mmol), after 45 min, gave product **39** (395.0 mg, 32%): mp 209-211 ºC; IR (KBr) ν 3370, 2971, 2833, 2180, 1629, 1367, 1271, 1240 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m/z*: $[M+1]^+$ 314.3; $[M+Na]^+$ 336.2; $[2M+1]^+$ 627.5; $[2M+Na]^+$ 649.5. Anal. Calcd. for $C_{17}H_{19}N_3O_3$: C, 65.16; H, 6.11; N, 13.41. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-4-(3'-methoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxylic acid (40). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (780.8 mg, 5.23 mmol) and ammonium acetate (1.21 g, 15.71 mmol), in methanol (15 mL), with compound **24** (1.0 g, 4.21 mmol), after 30 min, furnished product **40** (856 mg, 65%): mp 192-194 ºC; IR (KBr) ν 3401, 3340,

3224, 2992, 2833, 2182, 1657, 1624, 1489, 1367, 1255 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 314.3; $[M+Na]^+$ 336.2; $[2M+1]^+$ 627.5; $[2M+Na]^+$ 649.5. Anal. Calcd. for $C_{17}H_{19}N_3O_3$: C, 65.16; H, 6.11; N, 13.41. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-4-(4'-methoxyphenyl)-2-methyl-1,4-dihydropiridine-3-carboxylic acid (41). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (780.8 g, 5.23 mmol) and ammonium acetate (1.21 g, 15.71 mmol), in methanol (15 mL), with compound **26** (1.0 g, 4.21 mmol), after 30 min, afforded product **41** (987 mg, 74%): mp 188-190 ºC; IR (KBr) ν 3403, 3346, 3227, 2980, 2927, 2898, 2833, 2179, 1662, 1496, 1320 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 314.3; $[M+Na]^+$ 336.2; $[2M+1]^+$ 627.5; $[2M+Na]^+$ 649.5, Anal. Calcd. for $C_{17}H_{19}N_3O_3$: C, 65.16; H, 6.11; N, 13.41. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-4-(3',4'-dimethoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxylic acid (42). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (545.0 mg, 3.69 mmol) and ammonium acetate (915.9 mg, 14.69 mmol), in methanol (15 mL), with compound **26** (850.0 mg, 3.05 mmol), in 35 min, gave product **42** (797.0 mg, 77%): mp 166-168 ºC; IR (KBr) ν 3420, 3348, 3224, 2934, 2898, 2833, 2179, 1650, 1513, 1266 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 344.0; $[M+Na]^+$ 382.1; $[2M+1]^+$ 687.5; $[2M+Na]^+$ 709.3, Anal. Calcd. for $C_{18}H_{21}N_3O_4$: C, 62.96; H, 6.16; N, 12.24. Found: C, H, N.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-(3'-pyridyl)-1,4-dihydropiridine-3-carboxylic acid (43). Following the **General Method**, the reaction of

cyanoacetimidate hydrochloride (883.2 mg, 5.9 mmol) and ammonium acetate (1.37 g, 17.9 mmol), in methanol (15 mL), with compound **27** (1.0 g, 4.56 mmol), after 25 min, furnished product **43** (855 mg, 66%): mp 226-228 ºC; IR (KBr) ν 3401, 2971, 2920, 2182, 1661, 1491, 1327 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m/z*: $[M+1]^+$ 285.2; $[M+Na]^+$ 307.3; $[2M+1]^+$ 569.5; $[2M+Na]^+$ 591.5, Anal. Calcd. for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.07; H, 5.90; N, 20.02.

Ethyl ester of 6-amino-5-cyano-2-methyl-4-(4'-pyridyl)-1,4-dihydropiridine-3-carboxylic acid (44). Following the **General Method**, the reaction of cyanoacetimidate hydrochloride (816.01 mg, 5.47 mmol) and ammonium acetate (1.37 g, 17.78 mmol), in methanol (15 mL), with compound **28** (1.0 g, 4.56 mmol), after 30 min, gave product **44** (1.1 g, 85%): mp 252-254 ºC; IR (KBr) ν 3425, 3347, 3228, 2981, 2172, 1642, 1592, 1485, 1370, 1328, 1264, 1217 cm^{-1}; ^{1}H NMR (see Table 7, **Supporting Information**); ^{13}C NMR (see Table 8, **Supporting Information**); MS (API-ES+) *m/z*: $[M+1]^+$ 285.2; $[M+Na]^+$ 307.3; $[2M+1]^+$ 569.5; $[2M+Na]^+$ 591.5. Anal. Calcd. for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, H, N.

General method for the Friedländer reaction. Aluminium chloride (1.2-1.7 equiv) was suspended in dry 1,2-dichloroethane (10 mL) at rt under argon. The corresponding 4*H*-benzopyran (1 equiv) and cyclohexanone (1.2-1.7 equiv) were added. The reaction mixture was heated under reflux (10-24 h). When the reaction was over (tlc analysis), a mixture of THF/H_2O (1:1) was added at rt. An aqueous solution of sodium hydroxide (10%) was added dropwise to the mixture until the aqueous solution was basic. After stirring for 30 min, the mixture was extracted three times with dichloromethane. The organic layer was washed with

brine, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The resultant solid was purified by silica gel flash chromatography using methanol/dichloromethane mixtures as eluent to give pure compounds.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahydro-2-methyl-4-phenyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (1). Following the **General method for the Friedländer synthesis**, reaction of compound **31** (200 mg, 0.71 mmol) with $AlCl_3$ (128.12 mg, 1.05 mmol) and cyclohexanone (102.9 mg, 1.05 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 5 h, gave compound **1** (245.5 mg, 96%): mp 213-215 ºC; IR (KBr) ν 3411, 2932, 1664, 1634, 1448, 1242 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 364.2; $[M+Na]^+$ 386.1; $[2M+Na]^+$ 749.3. Anal. ($C_{22}H_{25}N_3O_2$) C, H, N.

Ethyl ester of 5-amino-4-(4'-fluorophenyl)-1,4,6,7,8,9-hexahydro-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (2). Following the **General method for the Friedländer synthesis**, reaction of compound **32** (200 mg, 0.66 mmol) with $AlCl_3$ (131.67 mg, 0.99 mmol) and cyclohexanone (97.02 mg, 0.99 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 10 h, gave product **2** (240 mg, 97%): mp 185-187 ºC; IR (KBr) ν 3416, 3369, 3231, 2978, 2933, 2862, 1663, 1633, 1603, 1574, 1449, 1382, 1269, 1244 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/z: $[M+1]^+$ 382.3; $[2M+Na]^+$ 763.7. Anal. ($C_{22}H_{24}FN_3O_2$) C, H, N.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahydro-2-methyl-4-(2'-nitrophenyl)-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (4). Following the **General method for the Friedländer synthesis**, reaction of compound **34** (200 mg, 0,61 mmol) with $AlCl_3$ (121.7 mg, 0.915 mmol) and cyclohexanone (89.72 mg, 0.915 mmol), in

$ClCH_2CH_2Cl$ (5 mL), after 6.5 h, gave product **4** (235.5 mg, 93%): mp 143-145 ºC; IR (KBr) ν 3391, 2932, 2855, 1619, 1526, 1449, 1233 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 409.2; $[2M+Na]^+$ 839.3. Anal. ($C_{22}H_{24}N_4O_4$) C, H, N.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahydro-2-methyl-4-(3'-nitrophenyl)-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (5). Following the **General method for the Friedländer synthesis**, reaction of compound **35** (200 mg, 0.61 mmol) with $AlCl_3$ (121.7 mg, 0.915 mmol) and cyclohexanone (89.7 mg, 0.91 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 5.2 h, gave product **5** (237.1 mg, 95%): pf 130-2 ºC; IR (KBr) ν 3415, 2932, 1681, 1634, 1612, 1574, 1528, 1449, 1351, 1224, 1100 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.20 (dd, 1 H, $J_{2'-4'}$= 2.0 Hz, $J_{2'-6'}$= 1.8 Hz, H2'), 8.03 (ddd, 1 H, $J_{4'-5'}$= 8.2 Hz, $J_{4'-2'}$= 2.0 Hz, $J_{4'-6'}$= 1.1 Hz, H4'), 7.66 (dd, 1 H, $J_{5'-6'}$= 7.9 Hz, $J_{4'-6'}$= 1.1 Hz, H6'), 7,40 (dd, 1 H, $J_{4'-5'}$= 8.2 Hz, $J_{5'-6'}$= 7.9 Hz, H5'), 6.71 [s, 1 H, NH(1)], 5.13 (s, 1 H, H4), 4.12 (q, 2 H, *J*= 7.1 Hz, $CO_2C\mathit{H}_2CH_3$), 4.01 (s, 2 H, NH_2), 2.68 (m, 2 H, H9), 2.39 [s, 3 H, $CH_3C(2)$], 2.32 (m, 2 H, H6), 1.80 (m, 4 H, H7, H8), 1.28 (t, 3 H, $CO_2CH_2C\mathit{H}_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.1 (C=O), 154.1 (C2), 148.9 (C3'), 148.0, 147.8, 147.7 (C9a, C10a, C5), 146.5 (C1'), 134.2 (C6'), 129.2 (C5'), 122.6 (C2'), 121.6 (C4'), 112.2 (C5a), 99.6, 99.0 (C3, C4a), 59.7 (OCH_2CH_3), 39.4 (C4), 32.2 (C9), 22.8 [$CH_3C(2)$], 22.4, 22.3 (C8, C6), 20.6 (C7), 14.3 (CH_3CH_2O); MS (API-ES+) *m*/*z*: $[M+1]^+$ 409.2; $[M+Na]^+$ 431.1; $[2M+Na]^+$ 839.3. Anal. ($C_{22}H_{24}N_4O_4$) C, H, N.

Ethyl ester of 5-amino-1,4,5,7,8,9-hexahydro-2-methyl-4-(4'-nitrophenyl) benzo[*b*][1,8]naphthyridine-3-carboxylic acid (6). Following the **General method for the Friedländer synthesis**, reaction of compound **36** (200 mg, 0.61 mmol) with $AlCl_3$ (122.0 mg, 0.91 mmol) and cyclohexanone (89.67 mg, 0.91 mmol), in

$ClCH_2CH_2Cl$ (5 mL), after 3.5 h, gave product **6** (225 mg, 94%): mp 201-203 ºC; IR (KBr) ν 3412, 2978, 2936, 2855, 1692, 1632, 1574, 1515, 1447, 1346, 1234 cm^{-1}; ^1H NMR (see Table 10, **Supporting Information**); ^{13}C NMR (see Table 9, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 409.2; $[2M+Na]^+$ 839.5. Anal. ($C_{22}H_{24}N_4O_4$) C, H, N.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahidro-2-methyl-4-(4'-methylphenyl)-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (7). Following the **General method for the Friedländer synthesis**, reaction of compound **37** (200 mg, 0.67 mmol) with $AlCl_3$ (134.22 mg, 1.01 mmol) and cyclohexanone (98.97 mg, 1.01 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 8.5 h, gave product **7** (235.5 mg, 92%): mp 201-203 ºC; IR (KBr) ν 3415, 3376, 2987, 2931, 2862, 1632, 1449, 1269, 1242 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 378.3; $[M+Na]^+$ 400.2; $[2M+1]^+$ 755.5. Anal. ($C_{23}H_{27}N_3O_2$) C, H, N.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahydro-4-(2'-methoxyphenyl)-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (9). Following the **General method for the Friedländer synthesis**, reaction of compound **39** (200 mg, 0.63 mmol) with $AlCl_3$ (127.28 mg, 0.96 mmol) and cyclohexanone (93.8 mg, 0.96 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 9.5 h, gave product **9** (161.5 mg, 64%): mp 196-198 ºC; IR (KBr) ν 3383, 2931, 1619, 1574, 1450, 1238 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 394.2; $[2M+Na]^+$ 809.5. Anal. ($C_{23}H_{27}N_3O_3$) C, H, N.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahydro-4-(3'-methoxyphenyl)-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (10). Following the **General**

method for the Friedländer synthesis, reaction of compound **40** (200 mg, 0.64 mmol) with $AlCl_3$ (127.28 mg, 0.96 mmol) and cyclohexanone (93.81 mg, 0.96 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 9.5 h, gave product **10** (240 mg, 95%): mp 156-158 ºC; IR (KBr) ν 3407, 3365, 3224, 2971, 2935, 2826, 1630, 1604, 1574, 1488, 1447, 1383, 1333, 1265, 1241 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 394.2; $[2M+Na]^+$ 809.7. Anal. ($C_{23}H_{27}N_3O_3$) C, H, N.

Ethyl ester of 5-amino-1,4,6,7,8,9-hexahydro-4-(4'-methoxyphenyl)-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid (11). Following the **General method for the Friedländer synthesis**, reaction of compound **41** (200 mg, 0.64 mmol) with $AlCl_3$ (127.28 mg, 0.96 mmol) and cyclohexanone (93.81 mg, 0.95 mmol), in $ClCH_2CH_2Cl$ (5 mL), after 9 h, gave product **11** (136 mg, 51%): mp 154-156 ºC; IR (KBr) ν 3407, 3362, 3224, 2978, 2930, 2862, 2826, 1663, 1631, 1602, 1574, 1508, 1449, 1302, 1242 cm^{-1}; ^1H NMR (see Table 9, **Supporting Information**); ^{13}C NMR (see Table 10, **Supporting Information**); MS (API-ES+) *m*/*z*: $[M+1]^+$ 394.2; $[2M+Na]^+$ 809.5. Anal. ($C_{23}H_{27}N_3O_3$) C, H, N.

Elemental analysis

Compound	Calcd.	Found
32 ($C_{16}H_{16}N_3O_2F$)	C, 63.78; H, 5.35 N, 13.95	C, 63.51; H, 5.27; N, 13.60
33 ($C_{17}H_{16}F_3N_3O_2$)	C, 58.12; H, 4.59 N, 11.96	C, 58.26; H, 4.35; N, 11.74
34 ($C_{16}H_{16}N_4O_4$)	C, 58.53; H, 4.91: N, 17.06	C, 58.76; H, 4.91; N, 17.12
35 ($C_{16}H_{16}N_4O_4$)	C, 58.53; H, 4.91: N, 17.06	C, 58.65; H, 4.89; N, 17.22
36 ($C_{16}H_{16}N_4O_4$)	C, 58.53; H, 4.91; N, 17.06	C, 58.35; H, 4.87; N, 16.86
37 ($C_{17}H_{19}N_3O_2$)	C, 68.67; H, 6.44; N, 14.13	C, 68.59; H, 6.33; N, 14.32
38 ($C_{22}H_{21}N_3O_2$)	C, 73.52; H, 5.89; N, 11.69	C, 73.27; H, 5.90; N, 11.83
39 ($C_{17}H_{19}N_3O_3$)	C, 65.16; H, 6.11; N, 13.41	C, 64.93; H, 6.40; N, 13.61
40 ($C_{17}H_{19}N_3O_3$)	C, 65.16; H, 6.11; N, 13.41	C, 65.36; H, 6.25; N, 13.70
41 ($C_{17}H_{19}N_3O_3$)	C, 65.16; H, 6.11; N, 13.41	C, 64.89; H, 5.97; N, 13.09
42 ($C_{18}H_{21}N_3O_4$)	C, 62.96; H, 6.16; N, 12.24	C, 62.69; H, 6.25; N, 12.43
43 ($C_{15}H_{16}N_4O_2$)	C, 63.37; H, 5.67; N, 19.71	C, 63.07; H, 5.90; N, 20.02
44 ($C_{15}H_{16}N_4O_2$)	C, 63.37; H, 5.67; N, 19.71	C, 63.24; H, 5.86; N, 19.70
1 ($C_{22}H_{25}N_3O_2$)	C, 72.70; H, 6.93; N, 11.56	72.71; H, 6.80; N, 11.53
2 ($C_{22}H_{24}FN_3O_2$)	C, 69.27; H, 6.34; N, 11.02	C, 69.47; H, 6.49; N, 10.96
3 ($C_{23}H_{24}F_3N_3O_2$)	C, 63.30; H, 5.31; N, 10.07	C, 63.60; H, 5.58; N, 9.89
4($C_{22}H_{24}N_4O_4$)	C, 64.69; H, 5.92; N, 13.72	C, 64.61; H, 6.04; N, 13.81
5 ($C_{22}H_{24}N_4O_4$)	C, 64.69; H, 5.92; N, 13.72	C, 64.87; H, 6.03; N, 13.55
6 ($C_{22}H_{24}N_4O_4$)	C, 64.69; H, 5.92; N, 13.72	C, 64.65; H, 5.86; N, 13.64
7 ($C_{23}H_{27}N_3O_2$)	C, 73.18; H, 7.21; N, 11.13	C, 73.27; H, 6.99; N, 11.40
8 ($C_{28}H_{29}N_3O_2$)	C, 76.51; H, 6.65; N, 9.56	C, 76.59; H, 6.71; N, 9.27
9 ($C_{23}H_{27}N_3O_3$)	C, 70.21; H, 6.92; N, 10.68	C, 69.92; H, 7.21; N, 10.44
10 ($C_{23}H_{27}N_3O_3$)	C, 70.21; H, 6.92; N, 10.68	C, 70.47; H, 6.79; N, 10.36

11 ($C_{23}H_{27}N_3O_3$)	C, 70.21; H, 6.92; N, 10.68	C, 70.33; H, 6.89; N, 10.58
12 ($C_{24}H_{29}N_3O_4$)	C, 68.06; H, 6.90; N, 9.92	C, 68.22; H, 6.70; N, 10.17
13 ($C_{23}H_{27}N_3O_3$)	C, 69.21; H, 6.64; N, 15.37	C, 69.27; H, 6.63; N, 15.28
14 ($C_{21}H_{24}N_4O_2$)	C, 69.21; H, 6.64; N, 15.37	C, 69,11; H, 6.69; N, 15.18

Table 7. ^{1}H NMR (DMSO-d_6, 300 MHz, δ) for compounds 32-44

	32	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**
NH[b]	8.67 (s)	8.70 (s)	8.76 (s)	8.74 (s)	8.79 (s)	9.03 (s)	8.08 (s)	8.57 (s)	8.63 (s)	8.68 (s)	8.90 (s)	8.74 (s)	8.77 (s)
NH_2[b]	5.68 (s)	5.62 (s)	5.76 (s)	5.84 (s)	5.83 (s)	5.77 (s)	5.74 (s)	5.49 (s)	5.68 (s)	5.66 (s)	5.74 (s)	5.77 (s)	5.81 (s)
H-4[b]	4.34 (s)	4.79 (s)	5.07 (s)	4.54 (s)	4.51 (s)	4.28 (s)	4.39 (s)	4.86 (s)	4.33 (s)	4.30 (s)	4.31 (s)	4.40 (s)	4.36 (s)
OCH_2CH_3	3.91 (q) *J*= 7.1	3.83 (c) *J*= 7.1	3.80 (c) *J*= 7.1	2.10 (c) *J*= 7.1	3.92 (c) *J*= 6.9	3.91 (c) *J*= 7.1	3.94 (c) *J*= 7.1	3.86 (c) *J*= 7.1	3.94 (c) *J*= 7.0	3.93 (c) *J*= 7.0	3.96 (c) *J*= 7.1	3.94 (c) *J*= 7.1	3.93 (c) *J*= 7.0
OCH_2CH_3	1.04 (t) *J*= 7.1	0.86 (t) *J*= 7.1	0.91 (t) *J*= 7.1	1.06 (t) *J*= 7.1	1.04 (t) *J*= 6.9	1.06 (t) *J*= 7.1	1.08 (t) *J*= 7.1	0.98 (t) *J*= 7.1	1.08 (t) *J*= 7.0	1.08 (t) *J*= 7.0	1.10 (t) *J*= 7.1	1.04 (t) *J*= 7.1	1.04 (t) *J*= 7.0
CH_3(C2)	2.24 (s)	2.28 (s)	2.25 (s)	2.29 (s)	2.29 (s)	2.21 (s)	2.27 (s)	2.28 (s)	2.26 (s)	2.24 (s)	2.25 (s)	2.28 (s)	2.29 (s)

[a] Multiplicity of signals are indicated in brackets , [b] Broad signal

(continued)

Table 7. ^{1}H NMR (DMSO-d_6, 300 MHz, δ) for compounds 32-44

	H3′	H5′	H2′	H6′	H4′	CH_3	OCH_3
32	7.10 (m)				-	-	
33	7.58 (m)	7.42 (m)	-	7.58 (m)	7.42 (m)	-	-
34	7.77 (d); $J_{3'-4'}$= 7.8	7.38 (t); $J_{5'-6'}$= 7.7	-	7.45 (d); $J_{6'-5'}$= 7.7	7.65 (t); $J_{4'-3'}$= 7.8		
35	-	7.62 (m)	7.95 (s)	7.62 (m)	8.07 (m)		
36	8.18 (d); $J_{2'-3'}$=8.2		8.18 (d); $J_{2'-3'}$=8.2		-	-	
37	7.00 (m)				-	2.22	
38	7.12 (m)	6.90 (m)	-	6.90 (m)	6.90 (m)		3.76 (s)
39	-	7.19 (t); *J*= 7.9	6.64 (s)	6.73 (d); *J*= 7.9			3.71(s)
40	6.82 (d); *J*= 8.6		7.04 (d); *J*= 8.6		-		3.70 (s)
41	-	6.86 (d) $J_{5'-6'}$= 8.3	6.71 (d) $J_{2'-6'}$= 2.0	6.64 $J_{6'-5'}$= 8.3; $J_{6'-2'}$= 2.0	-		3.70 (s)
42	-	8.38 (m)		7.50 (m)	7.30 (m)		
43	8.47 $J_{3'-6'}$= $J_{5'-2'}$= 1.5 $J_{3'-2'}$= $J_{5'-6'}$= 4.5		7.12 $J_{6'-3'}$= $J_{2'-5'}$= 1.5 $J_{2'-3'}$= $J_{6'-5'}$= 4.5		-	**Ph-C4′**	
44	7.60 (AB) 2HA; *J*= 18.8		7.60 (AB) 2HB; *J*= 18.8		-	7.43 (t) $J_{3''-4''}$= $J_{3''-2''}$= 7.5 7.32 (t) $J_{4''-3''}$= 7.5 7.20 (d) $J_{2''-3''}$= 7.5	

[a] Multiplicity of signals are indicated in brackets , [b] Broad signal

Table 8. ^{13}C **NMR (DMSO-d_6, 75 MHz, δ) of compounds 32-44**

	CO	C2	C6	CN	C3	C5	C4	OCH_2CH_3	CH_3
32	166.4	150.3	145.1	121.5	100.7	57.4	39.7	59.0; 13.9	18.6
33	166.8	151.8	146.6	121.0	101.4	58.2	35.9	59.2; 14.1	19.1
34	166.2	147.2	146.8	121.0	100.8	57.0	34.5	59.4; 14.1	19.1
35	166.5	150.9	146.5	121.4	100.3	57.0	39.8	59.6; 14.2	19.1
36	166.5	150.8	146.6	121.5	99.9	56.7	40.7	59.5;14.3	19.1
37	166.5	150.5	144.8	121.8	100.7	57.3	39.7	58.9; 14.0	18.6
38	166.5	150.4	145.2	121.7	100.6	57.2	39.8	59.1; 14.0	18.7
39	167.1	151.0	146.0	122.1	100.7	57.9	32.9	59.3; 14.3	19.0
40	166.5	150.4	145.0	121.7	100.7	57.3	40.0	59.0; 14.0	18.6
41	166.6	150.4	144.5	121.8	101.2	57.7	39.5	59.0; 14.0	18.6
42	166.6	150.4	147.2	121.8	100.9	57.3	39.7	58.9; 14.0	18.5
43	166.5	150.8	146.2	121.7	100.2	57.1	37.1	59.5; 14.3	19.0
44	166.5	155.9	151.0	121.6	99.6	56.4	40.4	59.5; 14.3	19.0

[*] **Interchangeable values**

(continued)

Table 8. ^{13}C NMR (DMSO-d_6, 75 MHz, δ) for compounds 32-44

	OCH_3	CH_3	C1'	C4´	C3´	C5´	C2´	C6´
32	-	-	143.96/143.93 $J_{C1'-F}$= 2.85	162.3/159.1 $J_{C1'-F}$= 240	114.9/114.6 $J_{C1'-F}$= 21.0		128.5/128.4 $J_{C1'-F}$= 240.0	
33	-	-	148.2	130.5*	125.28/125.2 125.13/125.0 $J_{C3'-F}$= 6.2	127.0	127.12/126.19 125.28/124.44 $J_{C2'-F}$= 65.8	133.3*
34	-	-	143.1	130.9	133.9	123.5	151.2	127.7
35	-	-	148.1	121.7	150.3	134.0	121.6	130.2
36	-	-	146.3	155.4	124.0		128.2	
37	-	20.6	135.0	144.8;	126.6		129.1	
38	-	-	146.9	138.1	127.3		128.8	
39	55.9	-	136.3	127.6*	128.3*	120.9*	156.3*	111.4*
40	54.8	-	145.0	110.8	159.1	129.2	112.9	119.0
41	54.9	-	139.1	157.7	113.5		127.7	
42	55.4; 55.3	-	148.2	140.4*	144.5*	111.8	110.8	118.6
43	-	-	148.4	147.8	-	124.0	143.2	134.6
44	-	-	146.7	-	150.0		122.2	

Table 9. ^{1}H NMR (DMSO-d_6, 300 MHz, δ) for compounds 1-4 and 6-14

	NH	NH_2 [b]	H4	H6[c]	H7[c]	H8[c]	H9[c]	OCH_2CH_3 [a]	CH3
1	9.14 (s)	5.26 (s)	4.99 (s)	2.16-2.05	1.65		2.53-2.22	3.97 (c);1.18 (t) *J*= 7.1	2.30 (s)
2	9.17 (s)	5.32 (s)	5.01 (s)	2.16-2.11	1.66		2.52-2.48	3.95 (c);1.15 (t) *J*= 7.1	2.27 (s)
3	9.36 (s)	5.43 (s)	4.91 (s)	2.33-2.07	1.65		2.56-2.52	4.03 (dc);1.08 (t) *J*= 7.1	2.14 (s)
4	9.46 (s)	5.70 (s)	5.53 (s)	2.34-2.17	1.60		2.57-2.48	3.94 (dc);1.14 (t) *J*= 7.1	2.29 (s)
6	9.31 (s)	5.47 (s)	5.20 (s)	2.12-2.07	1.67		2.56-2.59	3.95 (c);1.16 (t) *J*= 7.1	2.28 (s)
7	9.10 (s)	5.20 (s)	4.92 (s)	2.27-2.07	1.65		2.54-2.31	3.94 (c);1.16 (t) *J*= 7.1	2.17 (s)
8	9.19 (s)	5.35 (s)	5.02 (s)	2.14-2.06	1.66		2.53-2.32	3.98 (c);1.20 (t) *J*= 7.1	2.29 (s)
9	9.15 (s)	5.41 (s)	5.26 (s)	2.23-2.17	1.65		2.50-2.44	3.96 (c);1.03 (t) *J*= 7.1	2.33 (s)
10	9.13 (s)	5.28 (s)	4.97 (s)	2.17-2.11	1.66		2.52-2.48	3.95 (c);1.17 (t) *J*= 7.1	2.26 (s)
11	9.31 (s)	5.21 (s)	4.92 (s)	2.16-2.07	1.66		2.52-2.48	3.94 (c);1.16 (t) *J*= 7.1	2.25 (s)
12	9.08 (s)	5.30 (s)	4.91 (s)	2.20-2.09	1.66		2.50-2.42	3.97 (c);1.18 (t) *J= 7.1*	2.31 (s)
13	9.26 (s)	5.45 (s)	5.05 (s)	2.19-2.07	1.65		2.52-2.29	3.94 (c);1.14 (t) *J*= 7.1	2.29 (s)
14	9.27 (s)	5.47 (s)	5.07 (s)	2.16-2.07	1.66		2.53-2.39	3.96 (c);1.15 (t) *J*= 7.1	2.29 (s)

[a] Multiplicity of signals are indicated in brackets , [b] Broad signal [c] Multiplet

(continued)

Table 9. ^{1}H NMR (DMSO-d_6, 300 MHz, δ) for compounds 1-4 and 6-14

	OMe	Me	H2′	H6′	H3′	H5′	H4′
1[a]	-	-	7.31 (d); $J_{2'-3'}$= 7.1		7.15 [47]; $J_{3'-2'}$= 7.1; $J_{3'-4'}$= 7.4		7.05 (t); $J_{4'-3'}$= 7.4
2	-	-	7.32 (t); $J_{2'-3'}$= 8.5		6.96 (t); $J_{2'-3'}$= 8.5		-
3	-	-	-	7.51 (m)		7.32 (m)	7.51 (m)
4	-	-	-	7.73 [47] $J_{6'-4'}$= 1.1; $J_{6'-5'}$= 6.9	7.59 (dt) $J_{3'-5'}$= 1.1 $J_{3'-4'}$= 8.3	7.39 [47] $J_{5'-3'}$= 1.1; $J_{5'-6'}$= 6.9	7.34 (td) $J_{4'-6'}$= 1.1; $J_{4'-3'}$= 8.3
6	-	-	7.58 (d); $J_{2'-3'}$= 8.8		8.05 (d); $J_{3'-2'}$= 8.8		-
7	-	2.17 (s)	6.95 (d); $J_{2'-3'}$= 8.0		7.174 (d); $J_{3'-2'}$= 8.0		-
8	-	-	7.39 (m)				
9	3.90 (s)	-	-	7.12 (m)		6.88 (m)	
10	3.66 (s)	-	6.92 (d) $J_{6'-2'}$= 1.9	6.32 [47] $J_{6'-5'}$= 7.8; $J_{6'-2'}$= 1.9	-	7.06 [47] $J_{5'-6'}$= 7.8; $J_{5'-4'}$= 7.8	6.83 (d) $J_{4'-5'}$= 7.8
11	3.64 (s)	-	6.71 (d); $J_{2'-3'}$= 8.3		7.15 (d); $J_{3'-2'}$= 8.3		-
12	3.67(s); 3.63(s)	-	7.08 (d) $J_{2'-6'}$= 1.80	6.65 [47] $J_{6'-2'}$= 1.8; $J_{6'-5'}$= 8.3	-	6.72 (d) $J_{5'-6'}$= 8.3	-
13	-	-	8.57 (d) $J_{2'-6'}$= 1.7	7.61 (dt) $J_{6'-2'}$= 1.7; $J_{6'-5'}$= 7.8	-	7.18 [47] $J_{5'-4'}$= 4.8; $J_{5'-6'}$= 7.8	8.25 [47] $J_{4'-2'}$= 1.6; $J_{4'-5'}$= 4.8
14	-	-	7.28 [47] $J_{2'-3'}$= $J_{6'-5'}$= 4.6; $J_{2'-6'}$= $J_{3'-5'}$= 1.4		8.34 [47] $J_{3'-2'}$= $J_{3'-6'}$= 4.6; $J_{3'-5'}$= $J_{2'-6'}$= 1.4		-

Table 10. ^{13}C NMR (DMSO-d_6, 75 MHz, δ) for compounds 1-4 and 6-14

	CO	C9a	C2	C10a	C5	C4	C3	C5a	C4a	C6	C7	C8	C9	OCH_2 CH_3
1	166.9	151.9	149.5	147.8	147.0	37.8	98.1	110.8	99.7	22.9	22.4	22.3	31.8	58.5; 14.3
2	166.8	152.0	149.5	148.4	146.9	36.9	98.0	110.8	99.5	22.9	22.4	22.2	31.8	58.5; 14.2
3	166.7	149.8	148.7	147.7	146.5	34.7	98.9	111.6	100.5	26.6	23.2	22.6	32.1	58.9; 14.5
4	166.6	153.2	150.5	149.7	147.5	33.9	97.6	111.4	98.6	23.2	22.7	22.4	32.1	59.3; 14.3
6	166.5	152.5	149.7	149.3	146.8	37.7	96.8	111.0	98.5	22.9	22.3*	22.2*	31.8*	58.6; 14.2
7	167.2	149.2	148.4	147.3	144.9	37.7	98.5	111.2	100.2	23.3	22.8	22.6	32.2	58.8; 14.6
8	167.2	149.9	148.7	147.3	147.0	37.7	98.3	111.2	99.9	23.3	22.7	22.6	32.1	58.9; 14.6
9	166.7	149.8	148.8	146.5	136.5	31.6	98.1	110.1	100.3	22.8	22.3	22.2	30.4	58.3; 14.3
10	166.8	149.5	148.9	148.3	147.0	37.7	97.9	110.8	99.6	22.9	22.4	22.3	31.8	58.4; 14.2
11	166.9	151.8	147.8	149.4	146.8	36.8	98.37	110.7	100.0	22.9	22.4*	22.3*	31.8*	58.4; 14.2
12	166.8	152.0	149.9	148.2	148.0	37.5	98.7	111.1	110.3	23.3	22.8	22.6	32.1	58.8; 14.7
13	166.6	152.2	149.5	149.0	146.9	35.3	97.2	111.0	98.8	22.9	22.4	22.3	31.8	58.6; 14.2
14	166.6	152.3	149.8	149.4	146.9	37.1	96.6	111.0	98.3	22.9	22.3	22.2	31.8	58.6; 14.2

*Interchangeable values

(continued)

Table 10. ^{13}C NMR (DMSO-d$_6$, 75 MHz, δ) for compounds 1-4 and 6-14

	OMe	Me	C1´	C2´	C6´	C3´	C5´	C4´
1	-	-	147.4	127.6				125.7
2	-	-	143.6	129.3/129.2 $J_{C2'-F}$= 1.5 Hz		114.3/114.1 $J_{C3'-F}$= 15 Hz		162.1/158.8 $J_{C4'-F}$= 247.5
3[a]	-	-	153.1	125.02/124.64 124.26/123.87 $J_{C2'-F}$= 29.5	133.3	126.15/126.09/ 126.03/125.96 $J_{C3'-F}$= 5.2	127.3	131.8
4	-	-	147.6	142.0	123.7	134.5	131.6	127.9
6	-	-	154.9	128.7		123.2		145.6
7	-	22.9	152.2	128.6		127.9		135.0
8	-	-	152.2	129.1		128.5		138.5
9	54.7	-	151.5	153.8	110.4	129.5	121.4	127.1
10	54.7	-	151.9	114.2	119.9	158.6	128.7	110.3
11	54.8	-	139.7	128.5		113.0		157.3
12	55.8; 55.7	-	140.7	112.8	119.6	147.1	112.2	147.2
13	-	-	142.6	148.7	134.8	-	123.2	-
14	-	-	155.2	122.8		149.1		-

Biology. Methods.

Measurement of AChE activity from *Electrophorus electricus*. To assess the inhibitory activity of the compounds towards AChE, we followed the spectrophotometric method of Rappaport[44] using purified AChE from–Ee (*Electrophorus electricus*) and acetylcholine chloride (29.5 mM) as a substrate. The reaction took place in a final volume of 2.5 mL of an aqueous solution containing 0.78 U AChE and 1.9 mM *m*-nitrophenol to produce a yellow colour which is lost as a function of enzyme activity. Inhibition curves were made by incubating this mixture with the different compounds for 30 min; a sample without any compound was always present to determine the 100% of enzyme activity. After the 30 min incubation, the loss of yellow colour by *m*-nitrophenol was evaluated by measuring absorbance at 405 nm in a spectrophotometric plate reader (iEMS Reader MF, Labsystems). The concentration of compound that produces 50% AChE activity inhibition (IC_{50}) was calculated by transforming the values of absorbance to Rappaport enzymatic activity units extrapolated from a calibration curve previously obtained. Data are expressed as means ± S.E.M. of at least three different experiments in triplicate.

Measurement of AChE activity from hAChE. The inhibitory activity of the compounds towards hAChE was determined following the method of Ellman[45] using AChE from human serum and acetylthiocholine chloride (0.55 mM) as a substrate. The reaction took place in a final volume of 1 mL of a phosphate buffer solution at pH 7.2 containing 0.035 U of AChE and 0.25 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) which produces the yellow anion 5-thio-2-nitrobenzoic acid. Inhibition curves were made by incubating the reaction mixture with the different compounds for 15 min; a sample without any compound was always present to determine the 100% of enzymatic activity. After the 15 min incubation period, the production of colour, as an indication of enzymatic activity, was evaluated by measuring absorbance at 412 nm in a

spectrophotometer plate reader (iEMS Reader MF, Labsystems).

Measurement of hBuChE activity. The inhibitory activity of the compounds towards BuChE was determined following the method of Ellman[45] using BuChE from human serum and butyrylthiocholine chloride (5 mM) as a substrate. The reaction took place in a final volume of 1 mL of a phosphate buffer solution at pH 7.2 containing 0.035 U of BuChE and 0.25 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) which produces the yellow anion 5-thio-2-nitrobenzoic acid. Inhibition curves were made by incubating the assay mixture with the different compounds for 15 min; a sample without any compound was always present to determine the 100% of enzymatic activity. After the 15 min incubation period, the production of colour, as an indication of enzymatic activity, was evaluated by measuring absorbance at 412 nm in a spectrophotometric plate reader (iEMS Reader MF, Labsystems).

Kinetic analysis of the AChE inhibition. To obtain estimates of the competitive inhibition constant K_i, reciprocal plots of 1/V versus 1/[S] were constructed at relatively low concentration of the substrate acetylthiocholine (below 0.6 mM) by using Ellman's method[45]. The plots were assessed by a weighted least square analysis that assumed the variance of V to be a constant percentage of V for the entire data set. Slopes of these reciprocal plots were then plotted against the concentration of **11** (range 0 – 0.135 μM) in a weighted analysis and K_i was determined as the intercept on the negative x-axis. Data analysis was performed with GraphPad Prism 4.03 software (GraphPad Software Inc.).

Determination of the inhibitory effect on $A\beta_{40}$ aggregation induced by hAChE. The determination of the inhibitory potency of **11** against the hAChE-induced $A\beta_{40}$ aggregation was performed as reported.[8] In brief, aliquots of 2 μL of $A\beta_{40}$ (Bachem AG, Switzerland) peptide, were lyophilized from a 2 mg/mL 1,1,1,3,3,3-

hexafluoro-2-propanol (HFIP) solution and dissolved in DMSO at a final concentration of 230 μM. Samples were incubated for 24 h at room temperature in 0.215 M Na phosphate buffer (pH = 8.0). For coincubation experiments, aliquots of hAChE (2.30 μM, Aβ/hAChE ratio 100:1) and hAChE in the presence of **11** (100 μM) were added. Blanks containing $A\beta_{40}$, hAChE, $A\beta_{40}$ plus **11**, and hAChE in 0.215 M Na phosphate buffer (pH= 8.0) were prepared. The final volume of each vial was 20 μL. To quantify amyloid fibril formation, the thioflavin T fluorescence method was used.[60-62] After incubation, samples were diluted to a final volume of 2 mL with 50 mM glycine-NaOH buffer (pH= 8.5) containing 1.5 μM thioflavin T. A 300-seconds-time scan of fluorescence intensity was carried out (λ_{exc} = 446 nm; λ_{em} = 490 nm) by FP-6200 fluorometer (Jasco Europe), and values at plateau were averaged after subtracting the background fluorescence of 1.5 μM thioflavin T solution. The percent inhibition of the AChE induced aggregation due to the presence of **11** was calculated by the following expression: $100-(IF_i/IF_o \times 100)$ where IF_i and IF_o are the fluorescence intensities obtained for $A\beta_{40}$ plus hAChE in the presence and in the absence of **11**, respectively, minus the fluorescent intensities due to the respective blanks.

Determination of the inhibitory effect on the self-mediated $A\beta_{42}$ aggregation. As reported in a previously published protocol,[63] HFIP pretreated $A\beta_{42}$ samples (Bachem AG, Switzerland) were solubilized with a CH_3CN/Na_2CO_3/NaOH (48.4/48.4/3.2) mixture in order to have a stable stock solution ($[A\beta_{42}]$ = 500 μM). Experiments were performed by incubating the peptide in 10 mM phosphate buffer (pH= 8.0) containing 10 mM NaCl, at 30 °C for 24 h (final Aβ concentration 50 μM) with and without **11** (10 and 50 μM, Aβ/**11** = 5/1 and 1/1). Blanks containing **11** were also prepared and tested. To quantify amyloid fibrils formation, the thioflavin T fluorescence method was used.[60-62] After incubation, samples were diluted to a final

volume of 2.0 mL with 50 mM glycine-NaOH buffer (pH 8.5) containing 1.5 μM thioflavin T. A 300-seconds-time scan of fluorescence intensity was carried out (λ_{exc}= 446 nm; λ_{em}= 490 nm, FP-6200 fluorometer, Jasco Europe), and values at plateau were averaged after subtracting the background fluorescence of 1.5 μM thioflavin T solution. The fluorescence intensities were compared and the percent inhibition due to the presence of the inhibitor was calculated by the following formula: $100-(IF_i/IF_o \times 100)$ where IF_i and IF_o are the fluorescence intensities obtained for $A\beta_{42}$ in the presence and in the absence of inhibitor, respectively.

Culture of SH-SY5Y cells. SH-SY5Y cells, at passages between 3 and 16 after de-freezing, were maintained in a Dulbecco's modified Eagle's medium (DMEM) containing 15 non-essential amino-acids (NEAAs) and supplemented with 10% fetal calf serum (FCS), 1 mM glutamine, 50 units/ml penicillin and 50 μg/mL streptomycin (reagents from GIBCO, Madrid, Spain). Cultures were seeded into flasks containing supplemented medium and maintained at 37 ºC in 5% CO_2/humidified air. Stock cultures were passaged 1:4 twice weekly. For assays, SH-SY5Y cells were sub-cultured in 48 well plates at a seeding density of $2x10^5$ cells per well, or in 96 well plates at a seeding density of $8x10^4$ cells per well. For the cytotoxicity experiments, cells were treated with drugs before confluence, in DMEM free of serum.

Measurement of cytosolic Ca^{2+} concentrations. For these experiments, SH-SY5Y neuroblastoma cells were grown at confluence in 96-well black dishes. Cells were loaded with 4 μM fluo 4/AM for 1 h at 37 °C in DMEM. Then cells were washed twice with Krebs-Hepes solution and kept at room temperature for 30 min before the beginning of the experiment. Fluorescence was measured in a fluorescence microplate reader (FLUOstar Optima, BMG, Germany). Wavelengths of excitation and emission

were 485 and 520 nm respectively.

Measurement of lactic dehydrogenase (LDH) activity. Extracellular and intracellular LDH activity was spectrophotometrically measured using a Cytotoxicity Cell Death kit (Roche-Boehringer. Mannheim, Germany) according to the manufacturer's indications. Total LDH activity was defined as the sum of intracellular and extracellular LDH activity; released LDH was defined as the percentage of extracellular compared to total LDH activity.

***In vitro* Blood-Brain Barrier (BBB) permeation assay.** Prediction of the brain penetration was performed using a parallel artificial membrane permeation assay (PAMPA), in a similar manner as described previously.[56] Commercial drugs, phosphate buffered saline solution at pH 7.4 (PBS), and dodecane were purchased from Sigma, Aldrich, Acros, and Fluka. Ethanol was reagent grade from Merck. The Millex filter units (PVDF membrane, diameter 25 mm, pore size 0.45 μm) were acquired from Millipore. The porcine brain lipid (PBL) was obtained from Avanti Polar Lipids. The donor microplate was a 96-well filter plate (PVDF membrane, pore size 0.45 μm) and the acceptor microplate was an indented 96-well plate, both from Millipore. The acceptor 96-well microplate was filled with 180 μL of PBS: ethanol (80 : 20) and the filter surface of the donor microplate was impregnated with 4 μL of porcine brain lipid (PBL) in dodecane (20 mg mL^{-1}). Compounds were dissolved in PBS : ethanol (80 : 20) at 1 mg mL^{-1}, filtered through a Millex filter, and then added to the donor wells (180 μL). The donor filter plate was carefully put on the acceptor plate to form a sandwich, which was left undisturbed for 5 hours at 25 ºC. After incubation, the donor plate is carefully removed and the concentration of compounds in the acceptor wells was determined by UV spectroscopy. Every sample is analysed in four wells and at least in three independent runs, and the results are given as the mean ± standard deviation. In

each experiment, 18 quality control standards of known BBB permeability were included to validate the analysis set.

Molecular modeling. Docking was performed with the program rDock, which is an extension of the program RiboDock[64] using an empirical scoring function calibrated based on protein-ligand complexes.[65] The reliability of rDock was assessed by docking tacrine at the catalytic site of the *Torpedo californica* AChE and propidium at the peripheral site of the mouse AChE, taking advantage of the X-ray crystallographic structures of the two complexes (PDB entries 1ACJ and 1N5R).[47,48] Docking of compound **11** was performed using a structural model of the human enzyme used in our previous studies.[66] Superposition of the X-ray crystallographic structures confirmed unambiguously the structural similarity of the ligand binding sites. Water molecules were removed from the coordinates, and the docking volume was defined as the space within 10 Å of the ligands for both catalytic and peripheral binding sites. Before docking, the structure of the ligands was built up and energy minimized at the MP2/6-31G* level using Gaussian03.[67] Each compound was subjected to 100 docking runs, and the output docking modes were analyzed by visual inspection in conjunction with the docking scores.

The structural and dynamical stability of the best poses of (*R*)- and (*S*)-enantiomers of **11** were examined by combining molecular dynamics simulations and MM/PBSA computations using the program AMBER. In all cases simulations were performed using the same protocol adopted in our previous studies.[66] Briefly, the enzyme was immersed in a pre-equilibrated box of TIP3P[68] water molecules. The final systems contained the protein-ligand complex and around 16000 water molecules (c.a. 57300 atoms). After thermalization at 298 K, a series of 12 ns trajectories were sampled for the two enantiomers in the receptor-ligand complex. The system was simulated in the NPT ensemble using periodic boundary conditions and Ewald sums for treating long-range electrostatic

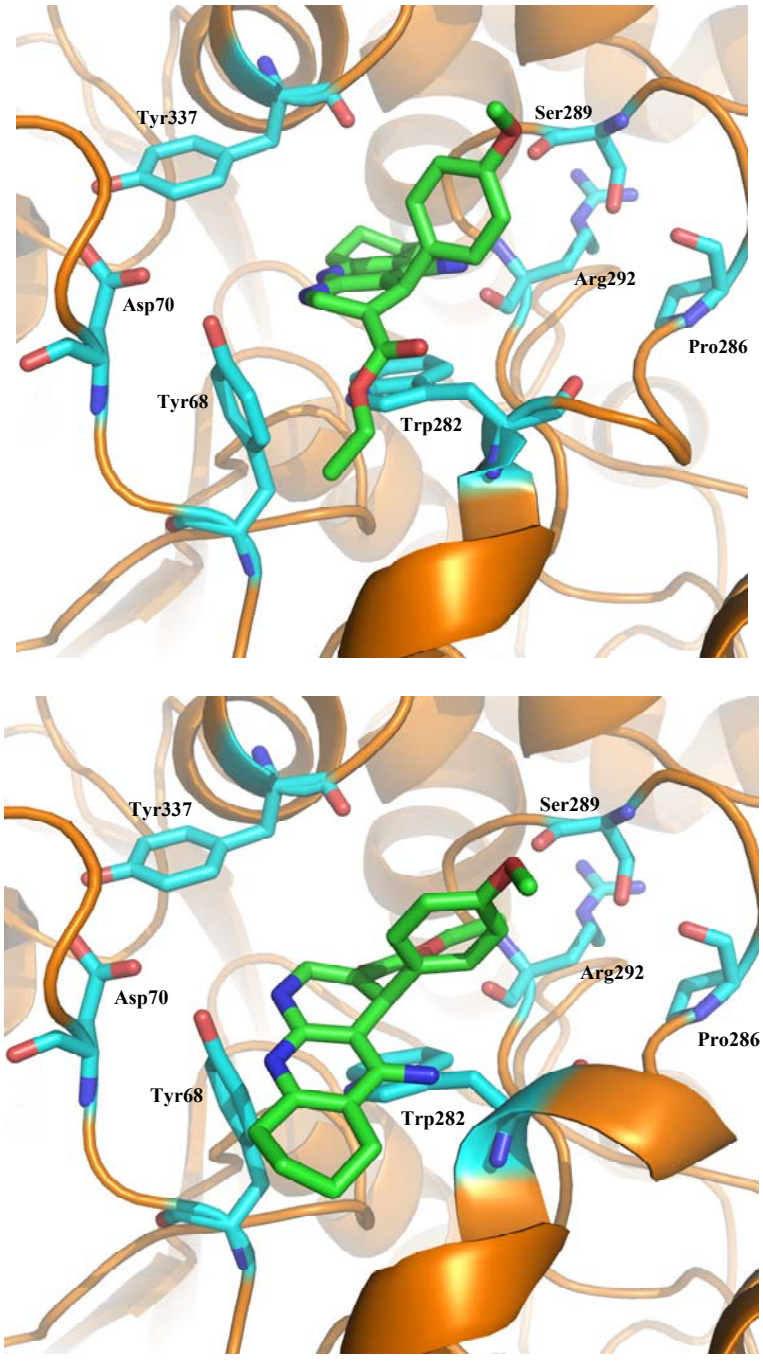
interactions (with the default Amber-9 parameters). All simulations were performed with the parmm99 force field of the Amber-9 package.[69]

The relative binding affinity of the *R*- and *S*-enantiomers was determined from the comparison of the stabilities of the two ligand-receptor complexes using Eq. 1.

$$\Delta G_{binding} = \Delta G_{MM} + \Delta G_{ele}^{sol} + \Delta G_{non-polar}^{sol} - T\Delta S \qquad \text{(eq 1)}$$

The partial atomic charges for the compounds were derived using the RESP protocol[70] by fitting to the molecular electrostatic potential calculated at the HF/6-31G* level with Gaussian-03. The internal conformational energy (ΔG_{MM}) was determined using the standard formalism and parameters implemented in AMBER. The electrostatic contribution (ΔG_{ele}^{sol}) was computed using a dielectric constant of 78.4 for the aqueous environment, while values of 2 and 4 were considered for the ligand-receptor complex. The electrostatic potentials were calculated using a grid-spacing of 0.25 Å. The interior of the solutes was defined as the volume inaccessible to a solvent probe sphere of radius 1.4 Å. The non-polar contribution ($\Delta G_{non-polar}^{sol}$) was calculated using a linear dependence with the solvent-accessible surface.[71] Finally, entropy changes upon complexation were assumed to be very similar in the two binding modes and therefore would cancel out in the comparison of the relative binding affinities. MM/PBSA computations were performed from the snapshots equally spaced at 50 ps intervals collected along the last 5 ns of the simulations, where all the free energy components reached converged values.

Fig. S1. Best pose of (top) (*R*)-11 and (bottom) (*S*)-11 enantiomers in the PAS from docking studies.



Chromatograms and experimental conditions for the chiral HPLC-mediated resolution of racemic "*p*-methoxytacripyrine 11", ^{1}H NMR spectra, quiroptical properties, and the inhibition of AChE/BuChE, of the separated enantiomers A and B.

Analytical experimental procedure. High Performance Liquid Chromatography with Ultraviolet and Mass detection (HPLC/MS) was used for the development of conditions for the resolution of the racemic mixture of *p*-methoxytacripyrine **11**, as well as for the determination of the enantiomeric excess (ee) of the two enantiomers isolated by semi-preparative chiral HPLC. Chromatographies were performed on CHIRALPAK® AD [amylose tris (3,5-dimethyl-phenyl carbamate)] units. The column dimensions were 150 x 4.6mm and 250 x 20 mm. for analytical and semi-preparative work, respectively. In both units, the enantioselective phase was coated onto silica-gel substrate (10 μm for semi-preparative scale and 5 μm for analytical studies). Experiments were carried out at room temperature. Flow rate was set up at 0.75 and 15 mL/min for analytical and semi-preparative work, respectively. Mobile phase consisted of 9/1, for analysis, and 98/2, for purification, methanol/acetonitrile mixtures (both solvents containing DMEA as basic additive and at 0.2% v/v). In all cases, the wavelength of UV detection was monitored from 200 to 400 nm although chromatograms were recorded at 254 and 280nm signals. Mass spectra were recorded using API-APCI ionization (full scan in positive/negative modes simultaneously). Retention time at analytical scale for the two target enantiomers are 4.87 and 8.27 min. Chiral quality control of isomers isolated by semi-preparative HPLC confirmed ee>98% for the two target compounds.

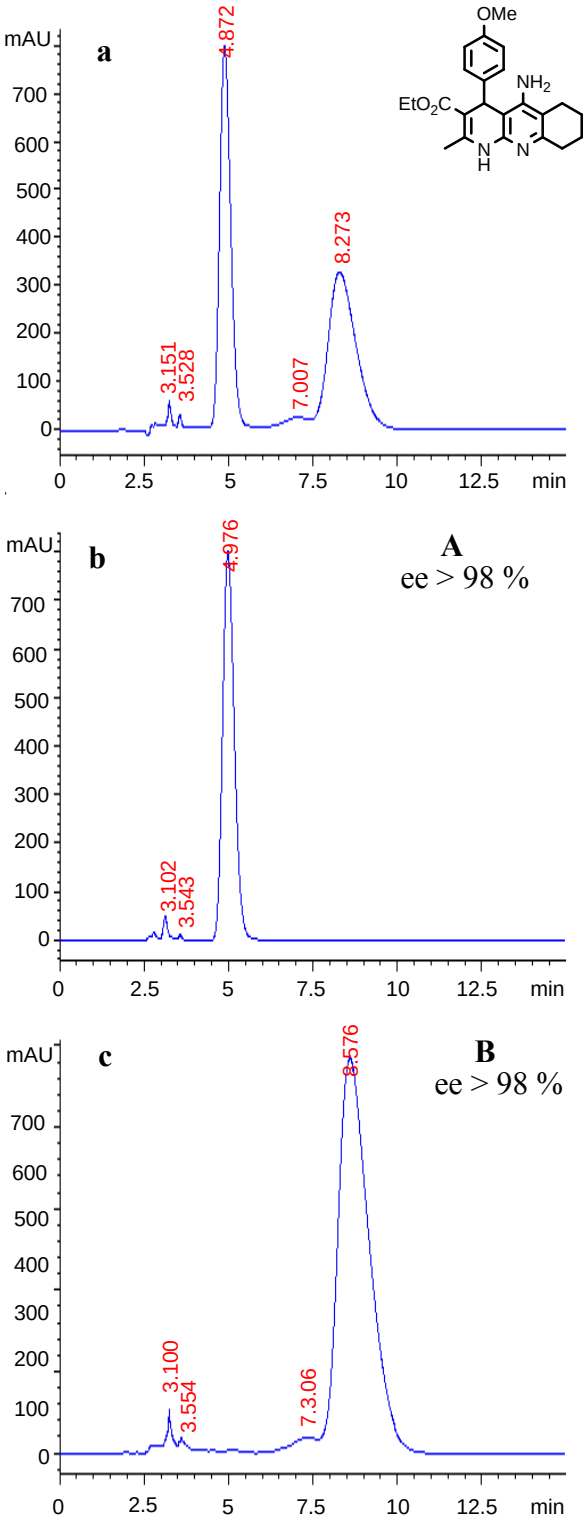
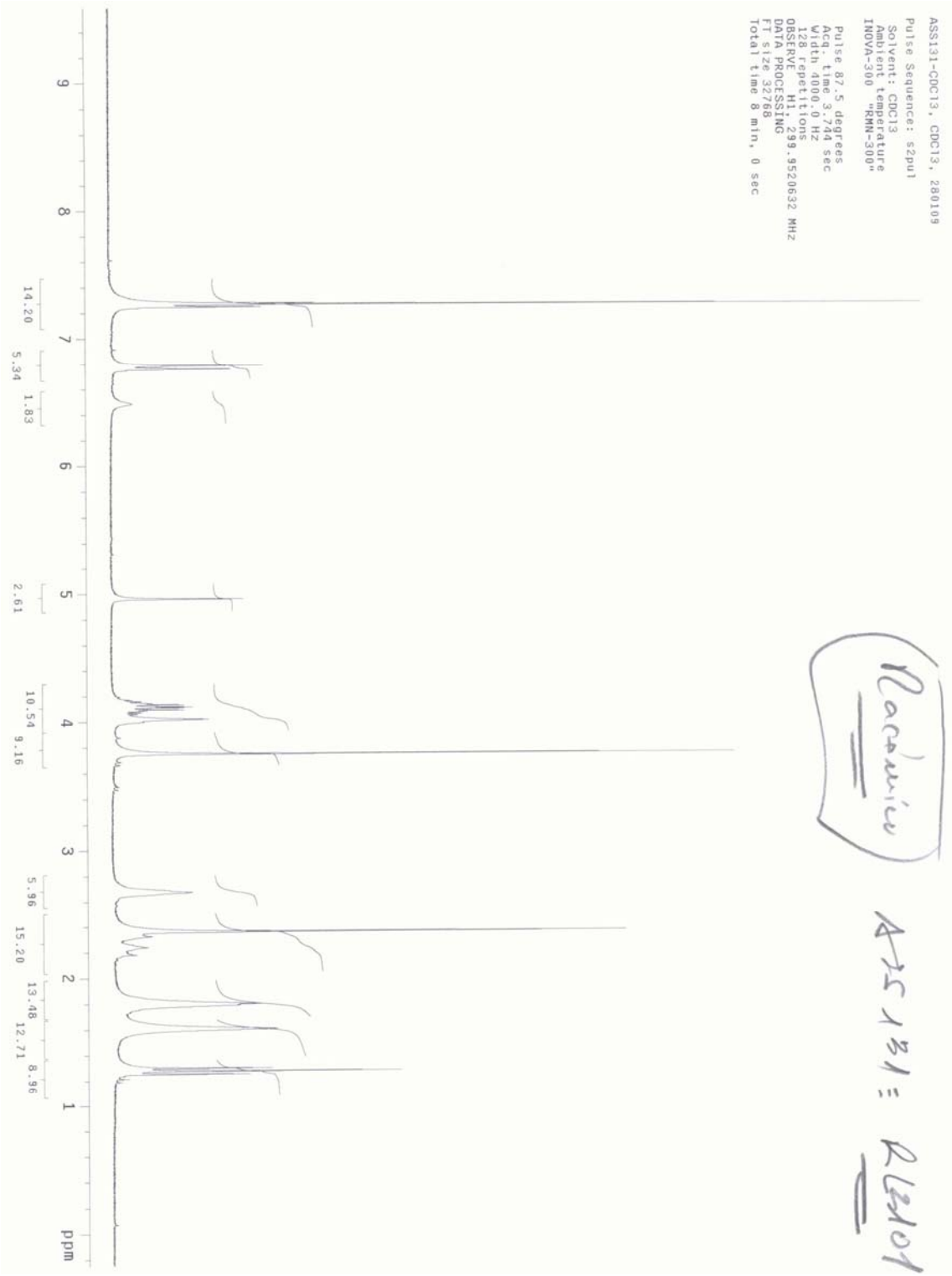
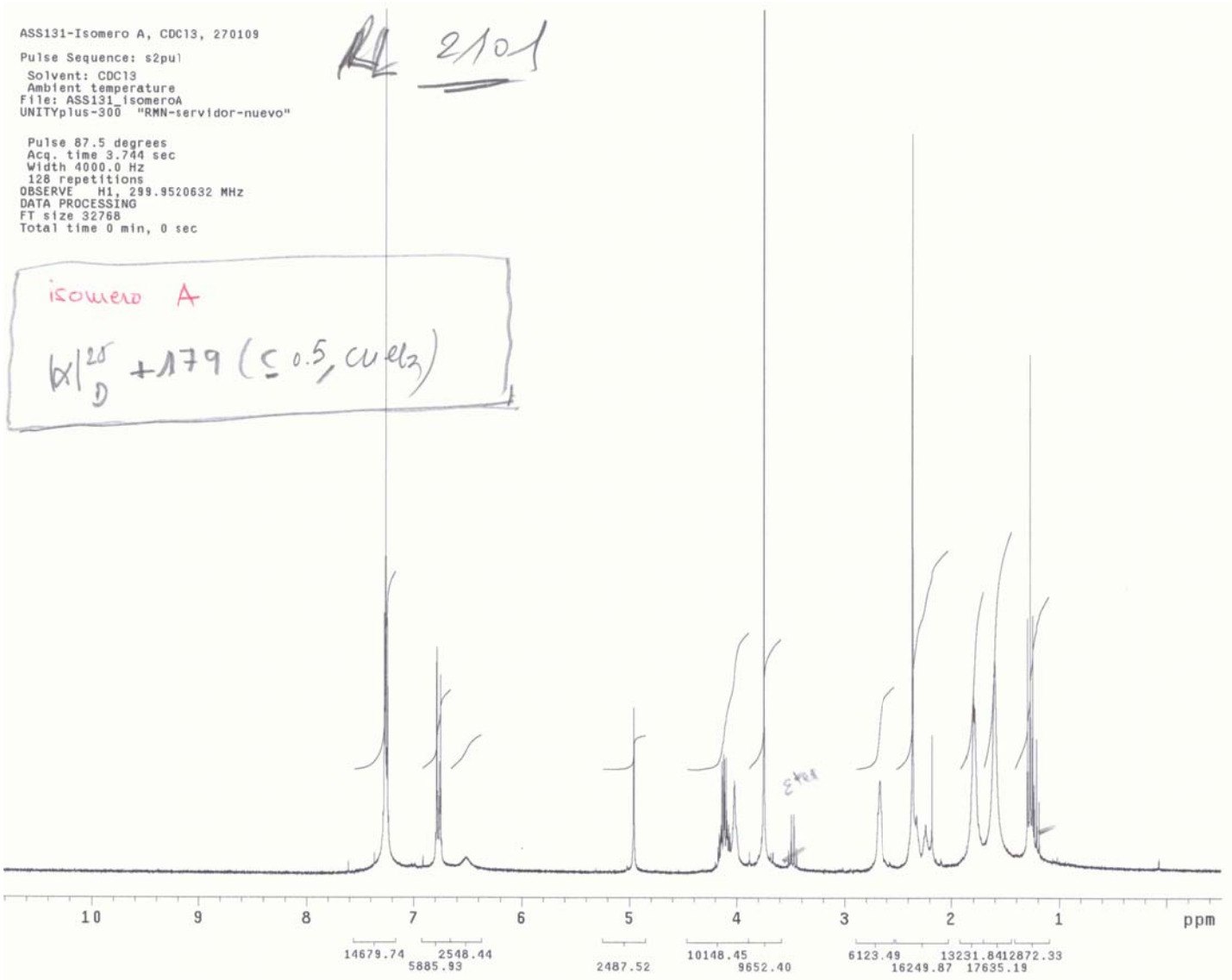


Fig. S2. Chromatograms of (**a**) the racemic *p*-methoxytacripyrine **11,** (**b**) enantiomer **A** and (**c**) enantiomer **B**.

^{1}H NMR spetcra of tacripyrine 11 (racemic, enantiomer A and enantiomer B)

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Solvent: CDCl3
Ambient temperature
File: ASS131_isomeroA
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Pulse 87.5 degrees
Acq. time 3.744 sec
Width 4000.0 Hz
128 repetitions
OBSERVE H1, 299.9520632 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
RL 2/10/1
isomero A
$[\alpha]_D^{25}$ +179 (c 0.5, CHCl3)
10
9
8
7
6
5
4
3
2
1
ppm
14679.74
2548.44
5885.93
2487.52
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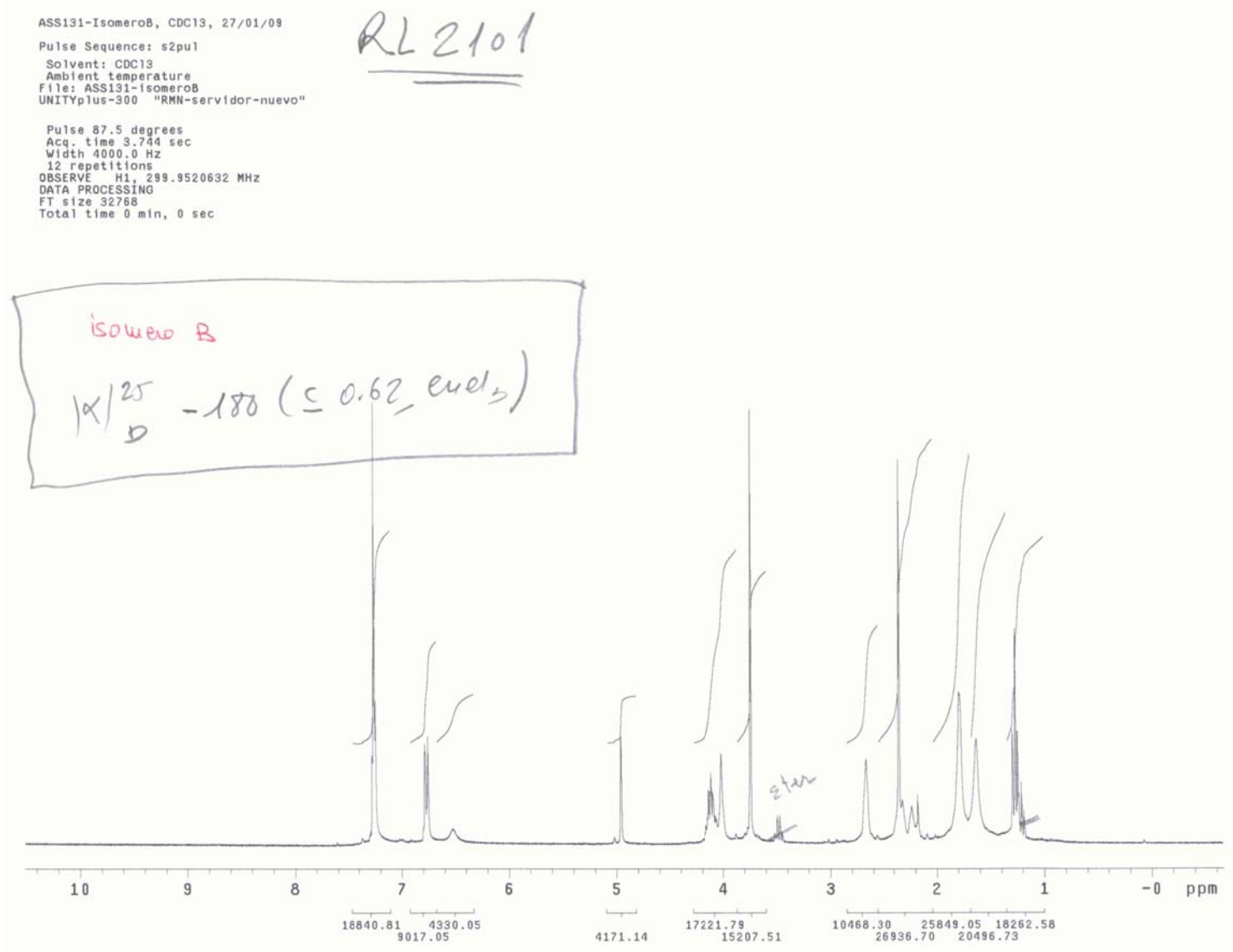
6123.49
13231.84
12872.33
16249.87
17635.19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Solvent: CDCl3
Ambient temperature
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Pulse 87.5 degrees
Acq. time 3.744 sec
Width 4000.0 Hz
12 repetitions
OBSERVE H1, 299.9520632 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
RL 2101
isomero B
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eter
10
9
8
7
6
5
4
3
2
1
-0
ppm
18840.81
4330.05
9017.05
4171.14
17221.79
15207.51
10468.30
25849.05
18262.58
26936.70
20496.73

Chiroptical properties

Isomer (**A**)-**11**: $[\alpha]_D$ + 179 (*c* 0.5, $CHCl_3$)

Isomer (**B**)-**11**: $[\alpha]_D$ −180 (*c* 0.62, $CHCl_3$)

Biological Activities

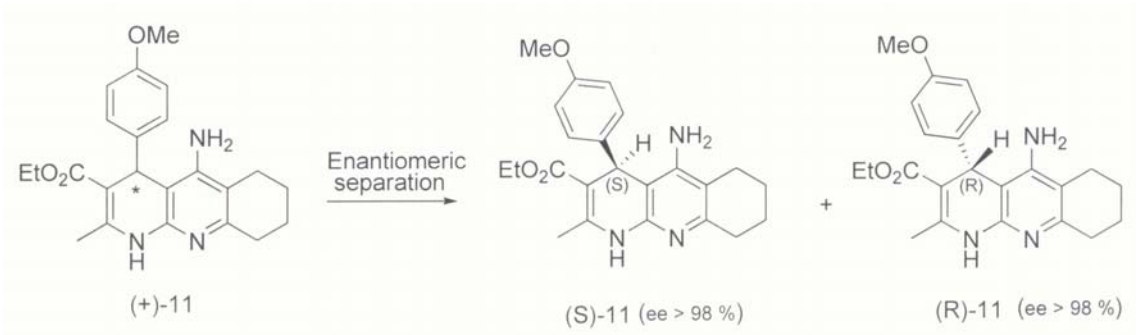

Table 11. Inhibition of AChE from *Electrophorus electricus* (EeAChE), human AChE (hAChE) and human BuChE (hBuChE) by racemic *p*-methoxytacripyrine **11** and their enantiomers (**A**)-**11** and (**B**)-**11**.

	IC_{50} (nM)[a] eeAChE[b]		IC_{50} (nM)[a] hBuChE[c]	IC_{50} (nM)[a] hAChE[c]	Selectivity[e] IC_{50} hBuChE/IC_{50} hAChE
	Rappaport method	Ellman method			
Tacrine	180 ± 20	**130 ± 10**	36 ± 4	147 ± 11	0.24
(±)-11	45 ± 5	nd	>100000	**105 ± 15**	>952
(A)-11	nd	**80 ± 12**	11000 ± 2000	**378 ± 40**	29
(B)-11	nd	**9 ± 1**	>100000	**36 ± 3**	2778

[a]IC_{50} values are the mean ± SEM of at least three independent measurements. [b]From *Electrophorus electricus*. [c]From human serum. [e]Human AChE and BuChE. nd: not determined.

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