Supplementary Material

Design, synthesis and biological evaluation of edaravone derivatives bearing the N-benzyl pyridinium moiety as multifunctional anti-Alzheimer’s agents
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¹H and ¹³C NMR discussion

The NMR peaks of compound 3 and 5a – 5l were observed on all ¹H NMR spectra except for the CH₂ within the pyrazoline ring of edaravone. Previous research has shown that this CH₂ peak is found at δ = ~ 3.4 ppm. Therefore, the peak is overlapped by the DMSO-d6 water peak that is found at the same chemical shift. The rest of the ¹H NMR peaks correlate to the proposed final compounds and HRMS confirmed their molecular masses.

Edaravone has three neutral tautomeric forms. In all ¹H NMR spectra a peak is visible at δ = ~ 5.4 ppm. This peak belongs to the amine tautomer of the edaravone derivative when dissolved in DMSO-d6. The NH peak of the amine tautomer at δ = ~ 11.7 ppm, was observed as a broad peak with an integration around 0.1. A further test was to determine which tautomeric form was most stable in MeOH-d4. It was discovered that the keto tautomer was more stable in methanol-d4 and no peak was present at δ = 5.4 ppm or δ = 11.7 ppm. The CH₂ group of the pyrazoline ring of edaravone is overlapped by the methanol-d4 solvent peak on the ¹H NMR spectra (δ = 3.3 ppm) and was therefore not observed. The experiment was also attempted in CDCl₃; however, these compounds are not soluble in this solvent system.

The NH peak of the amide linker is present on all DMSO-d6 ¹H NMR spectra at δ = ~9.3 ppm. The integration of the NH peak did not account for one proton. The reason is that the proton of NH is a labile proton. The integration is affected by possible hydrogen bonding taking place between the amide and water found in the DMSO-d6. To confirm that the NH of the amide was a labile, an ¹H NMR experiment was conducted in methanol-d4. Methanol-d4 is a protic solvent and therefore MeOD/H exchanges with the deuterium atom of MeOD and becomes ND. Deuterium resonates at 61 MHz on a 400 MHz instrument whereas ¹H resonates at 400MHz. Therefore, the ND is in a different window and cannot be observed on the 400 MHz spectra. As DMSO-d6 is an aprotic solvent this H exchange does not take place. A disappearance of the NH peak, of the amide linker, was observed when the ¹H NMR was conducted in methanol-d4. It was further confirmed
with HSQC NMR as no carbon correlated with this peak. Therefore, the presence of the secondary amine of the amide was confirmed.

\(^{13}\text{C}\) NMR was also conducted and it was observed that the methyl peak at around \(\delta = 17.1\) ppm was not present on all \(^{13}\text{C}\) NMR spectra. It was found that when examining the HSQC NMR spectra two \(^{13}\text{C}\) peaks at \(\delta = 17.1\) ppm and \(\delta = 14.6\) ppm correlated with the methyl peak on the \(^1\text{H}\) NMR at a \(\delta = 2.1\) ppm. The two carbons correlating to the one proton could be caused by the spinning of the methyl group. Thus, this shows that a methyl group is present. \(^1\text{H}\) NMR as well as MS further confirms the above reasoning.

**HRMS discussion**

The molecular masses were confirmed with HRMS. Compound 3 exhibited a \([\text{M+H}]^+\) peak. Compounds 5a-l all exhibited \([\text{M-Br}]^+\) peaks. The bromine is pulled away from the structure and can be observed in spectrum at \(m/z= 79.0220\) amu. It was also be observed that both chlorine (5e-f) and bromide (5g-i) substituted compounds have a \([\text{M+2H}]^+\) peaks. This is due to isotopic abundance of these two halogens. Bromine comes in two isotopes that have respective molecular weights of 79 g/mol and 81 g/mol. The natural abundance of these two isotopes are 50.69% and 49.31% respectively. Therefore, two peaks on the MS spectra of 5g-i that are approximately the same peak height and 2 amu apart is observed. Chlorine also has two isotopes that have respective molecular weights of 35 g/mol and 37 g/mol. The natural abundance of these isotopes is 75.78% and 24.22% respectively. Therefore, two peaks on the MS spectra of 5e-f with a peak height of 1 \((^{35}\text{Cl}): 0.33 (^{37}\text{Cl})\) and 2 g/mol apart is observed.
**Molecular docking studies**

**Table 1**: 3D and 2D representations and interactions of compound 3 and 5a-5l docked within TcAChE active site.

| Comp. | Ligand interactions with aromatic residues (2D) | Compounds docked within the TcAChE active site (3D) |
|-------|-----------------------------------------------|---------------------------------------------------|
| 3     | ![2D representation of 3](image1)             | ![3D representation of 3](image2)                |
| 5a    | ![2D representation of 5a](image3)           | ![3D representation of 5a](image4)               |
| 5b    | ![2D representation of 5b](image5)           | ![3D representation of 5b](image6)               |
| 5c    | ![2D representation of 5c](image7)           | ![3D representation of 5c](image8)               |
AChE inhibition dose response curves

$\text{IC}_{50} = >100 \ \mu \text{M}$

$\text{IC}_{50} = 30 \ \mu \text{M}$

$\text{IC}_{50} = 1.2 \ \mu \text{M}$

$\text{IC}_{50} = 4.6 \ \mu \text{M}$

$\text{IC}_{50} = 3.6 \ \mu \text{M}$

$\text{IC}_{50} = 1.9 \ \mu \text{M}$

$\text{IC}_{50} = 3.3 \ \mu \text{M}$

$\text{IC}_{50} = 3.5 \ \mu \text{M}$
$IC_{50} = 11.5 \ \mu M$

$IC_{50} = 69.1 \ \mu M$

$IC_{50} = 19.9 \ \mu M$

$IC_{50} = 95.5 \ \mu M$
BuChE inhibition dose response curves

3

$IC_{50} > 1000 \mu M$

5a

$IC_{50} > 1000 \mu M$

5b

$IC_{50} > 1000 \mu M$

5c

$IC_{50} = 926 \mu M$

5d

$IC_{50} > 1000 \mu M$

5e

$IC_{50} = 890 \mu M$

5f

$IC_{50} = 891 \mu M$

5g

$IC_{50} = 160 \mu M$
IC₅₀ = >1000 μM

IC₅₀ = 630 μM

IC₅₀ = >1000 μM

IC₅₀ = >1000 μM
Antioxidant activity (DPPH⁺) dose response curves

1

% Inhibition

Log [M]

IC₅₀ = 45.7 µM

3

% Inhibition

Log [M]

IC₅₀ = 12.5 µM

5a

% Inhibition

Log [M]

IC₅₀ = 22.9 µM

5b

% Inhibition

Log [M]

IC₅₀ = 28.8 µM

5c

% Inhibition

Log [M]

IC₅₀ = 25 µM

5d

% Inhibition

Log [M]

IC₅₀ = 11.5 µM

5e

% Inhibition

Log [M]

IC₅₀ = 13.8 µM

5f

% Inhibition

Log [M]

IC₅₀ = 13.1 µM
**Spectral Data**

$^1$H NMR; $^{13}$C NMR; HSQC NMR; IR; MS
Compound 3

Spectrum 1: $^1$H NMR Compound 3

"Compound 3" 1 1 "/Users/lukezondagh/Desktop/lab work/NMR"

PROTON DMSO {C:/Bruker/TopSpin3.2} JJ-LukeZondagh 15
Spectrum 2: $^{13}$C NMR Compound 3

"Compound 3" 2 1 "\text{\textasciitilde/Users/lukezondagh/Desktop/lab work/NMR}"

$\text{C13CPD DMSO \{C:\text{\textasciitildeBruker\textbackslash TopSpin3.2\textbackslash JJ-LukeZondagh 15\} \]}

166.3652 149.9808 128.6892 122.6220
Spectrum 3: HSQC NMR Compound 3

"Compound 3" 6 1 "/Users/lukezondagh/Desktop/lab work/NMR"

HSQCEDETGPSISP2.3
HSQCEDETGPSISP DMSO {C:\Bruker\TopSpin3.2} JJ-LukeZondagh 2
Spectrum 4: IR Compound 3

%T

48.0 60 70 80 90 98.0

4000.0 3000 2000 1500 1000 650.0 cm⁻¹

3217.38 3035.21 1713.55 1603.24 1562.27 1500.94 1401.19 1357.76 1306.29 1294.34 1195.34 1145.23 1056.62 1016.46 994.45 959.56 904.45 884.42 852.66 796.38 679.27

699.45 731.78 754.91 770.87 832.44 884.42 679.27
Spectrum 5: MS Compound 3
Spectrum 6: $^1$H NMR Compound 5a
Spectrum 7: $^{13}$C NMR Compound 5a

"Compound 5a" 2 1 /Users/lukezondagh/Downloads

$^1$H NMR DMSO [C:\Bruker\TopSpin3.6.2]\-LukeZondagh 19

[Graph of the $^{13}$C NMR spectrum with peaks at various ppm values]
Spectrum 9: MS Compound 5a

Comp 5a

MS_Direct_191210_21 21 (0.133) Cm (13:22) 1: TOF MS ES+ 1.50e7

m/z 399.1820 400.1847 401.1876 413.1609
Compound 5b

Spectrum 10: $^1$H NMR Compound 5b
Spectrum 11: $^{13}$C NMR Compound 5b
Spectrum 12: IR Compound 5b
Spectrum 13: MS Compound 5b

Comp 5b
MS_Direct_191210_20 27 (0.175) Cm (25:37)

1: TOF MS ES+
2.63e6
417.1719
413.1969
418.1752
433.1451
419.1779
466.2782
465.2749
466.2782
Compound 5c

Spectrum 14: $^1$H NMR Compound 5c
Spectrum 15: $^{13}$C NMR Compound 5c
Spectrum 16: IR Compound 5c
Spectrum 17: MS Compound 5c
Spectrum 18: $^1$H NMR Compound 5d
Spectrum 19: $^{13}$C NMR Compound 5d
Spectrum 20: IR Compound 5d

%T

97.7
95
90
85
80
75
70
65
60.4

4000.0 3000 2000 1500 1000 650.0 cm-1

3201.22 3036.90 3147.59 1716.72 1643.38 1603.84 1562.00 1536.70 1502.58 1454.49 1405.74 1397.34 1356.12 1325.08 1297.02 1283.15 1228.19 1183.15 1151.05 1125.08 1100.68 1034.02 1015.42 980.95 918.00 855.32 800.49 767.78 731.97 689.98 631.97 587.78 531.97 487.78 431.97 387.78 331.97 287.78 231.97 187.78 131.97 87.78 cm-1

3201.22 3036.90

60.4
Spectrum 21: MS Compound 5d
Spectrum 22: $^1$H NMR Compound 5e
Spectrum 23: \textsuperscript{13}C NMR Compound 5e
Spectrum 25: MS Compound 5e
Compound 5f

Spectrum 26: $^1$H NMR Compound 5f
Spectrum 27: $^{13}$C NMR Compound 5f

"Compound 5f" 21 /Users/lukezondagh/Downloads
C13CPD DMSO {C:\Bruker\TopSpin3.2} JJ-LukeZondagh
Spectrum 28: HSQC NMR Compound 5f
Spectrum 29: IR Compound 5f

%T

4000.0 3000 2000 1500 1000 650.0

54.3

65

70

75

80

85

90

96.8



cm⁻¹

3213.83

3035.24

1710.98

1639.10

1604.35

1563.37

1536.65

1496.80

1468.85

1402.61

1358.18

1306.06

1200.12

1143.82

1083.32

1056.17

997.74

981.10

903.55

884.16

801.57

755.36

732.18

690.81

771.20

662.96

543.0

4000.0 1500 1000 650.0
Spectrum 30: MS Compound 5f

Comp 5f
MS_Direct_191210_17 45 (0.262) Cm (45:49)

m/z
360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470

1: TOF MS ES+
1.81e6 433.1430
413.1971 372.0557
435.1410
436.1437
465.2755 447.1224 437.1462
449.1251
466.2782
Compound 5g

Spectrum 31: $^1$H NMR Compound 5g
Spectrum 34: IR Compound 5g

%T vs. cm⁻¹

- 3217.64
- 3034.57
- 1711.00
- 1566.98
- 1536.34
- 1505.61
- 1496.86
- 1482.55
- 1469.03
- 1458.22
- 1402.55
- 1358.22
- 1305.61
- 1200.37
- 1083.12
- 1034.85
- 981.23
- 967.00
- 883.95
- 857.29
- 801.75
- 771.02
- 755.00
- 732.05
- 721.02
- 690.61
- 662.87
- 643.00
- 614.50
- 586.20
- 555.00
- 524.00
- 494.00
- 464.00
- 434.00
- 404.00
- 374.00
- 344.00
- 314.00
- 284.00
- 254.00
- 224.00
- 194.00
- 164.00
- 134.00
- 104.00
- 74.00
- 44.00
- 14.00
- 0.0

Wavenumbers (cm⁻¹)
Spectrum 35: MS Compound 5g
Spectrum 36: $^1$H NMR Compound 5h
Spectrum 37: $^{13}$C NMR Compound 5h

"Compound Si" 3 1 /Users/lukezondag/Downloads

$C_{13}CPD$ DMSO {C:\Bruker\TopSpin3.6.2\} J=LukeZondagh 18
Compound 5i

Spectrum 40: $^1$H NMR Compound 5i
Spectrum 41: $^{13}$C NMR Compound 5i
Spectrum 42: IR Compound 5i

- Wavenumbers (cm⁻¹): 3217.61, 3034.52, 1715.52, 1638.73, 1603.99, 1562.30, 1536.53, 1497.01, 1401.58, 1356.99, 1197.40, 1144.12, 1056.70, 997.25, 980.96, 884.53, 856.48, 800.95, 770.88, 690.49, 662.37
- %T: 96.8, 90, 85, 80, 75, 70, 65, 60, 57.4

- Wavenumber range: 4000.0 - 650.0 cm⁻¹

- %T range: 96.8 - 57.4
Spectrum 43: MS Compound 5i

Comp 5i
MS_Direct_191210_14 22 (0.137) Cm (19:24)

1: TOF MS ES+
3.18e6
479.0926
477.0943
465.2766
461.9535
459.9553
466.2791
480.0952
481.0978
493.0746
491.0729
Compound 5j

Spectrum 44: $^1$H NMR Compound 5j
| cm$^{-1}$ | %T  |
|---------|-----|
| 4000.0  | 60.3 |
| 3000.0  | 65  |
| 2000.0  | 70  |
| 1500.0  | 75  |
| 1000.0  | 80  |
| 650.0   | 85  |

Spectrum 46: IR Compound 5j
Spectrum 47: MS Compound 5j

Comp 5K
MS_Direct_191210_13 19 (0.126) Cm (14:19)

1: TOF MS ES+
2.58e6
413.1968
414.2002
415.2028
Spectrum 48: $^1$H NMR Compound 5k
Spectrum 49: $^{13}$C NMR Compound 5k

 Compound SL 2 1 /Users/lukezondagh/Downloads
 C13CPD DMSO {C:\Bruker\TopSpin3.2} Jf-LukeZondagh 2

166.675 160.679 144.7695 138.1082 134.7669 130.6581 129.6588 128.8583 126.3805 63.1912 42.8170 40.4109 40.4109 32.6578 21.3813

[ppm]
Spectrum 50: HSQC NMR compound 5k

"Compound SJ" 4 1 /Users/lukezondagh/Downloads
HSQCEDETCPSPISP2.3
HSQCEDETCPSPISP DMSO {C:\Bruker\TopSpin3.2\J-LukeZondagh 2
Spectrum 52: MS Compound 5k
Compound 51

Spectrum 53: $^1$H NMR Compound 51
Spectrum 55: HSQC NMR Compound 51
Spectrum 57: MS Compound 51

1: TOF MS ES+ 1.37e5

M/z 309.1347 413.1974 414.2005 415.2016 517.2576