Supporting Information

Dual-acting monoamine oxidase-B and acetylcholinesterase inhibitors containing the morpholine group: Synthesis and biochemical investigations

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Spectral Characterization of the synthesized compounds

(2E)-1-[4-(morpholin-4-yl)phenyl]-3-phenylprop-2-en-1-one (MO1): $^1$H-NMR (500 MHz, DMSO) $\delta$: 3.34–3.33 (4H, t, J = 5.0 Hz, morpholine -N-(CH$_2$)$_2$), 3.75–3.74 (4H, t, J = 5.0 Hz, morpholine O-(CH$_2$)$_2$), 7.04–7.02 (2H, d, H3’ & H5’), 7.45–7.43 (3H, m, H3, H4 & H5), 7.69–7.65 (1H, d, J = 15.0 Hz, -CH$\alpha$), 7.88–7.87 (2H, d, H2&H6), 7.94–7.91 (1H, d, J = 15.0 Hz, -CH$\beta$), 8.09–8.07 (2H, d, H2’&H6’). $^{13}$C-NMR (500 MHz, DMSO) $\delta$: 186.46, 153.99, 142.14, 134.90, 130.46, 130.10, 128.76, 128.53, 127.39, 122.10, 112.97, 65.75, 46.62. ESI-MS ($m/z$): Calculated- 293.3596, Observed-293.3593.

(2E)-3-[4-hydroxyphenyl]-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO2): $^1$H-NMR (500 MHz, DMSO) $\delta$: 3.32–3.31 (4H, t, J = 5.0 Hz, morpholine -N-(CH$_2$)$_2$), 3.74–3.73 (4H, t, J = 5.0 Hz, morpholine O-(CH$_2$)$_2$), 6.43 (1H, s, Ar-OH), 7.03–7.01 (2H, d, H3’ & H5’), 7.46–7.44 (2H, m, H3 & H5), 7.66–7.63 (1H, d, J = 15.0 Hz, -CH$\alpha$), 7.87–7.86 (2H, d, H2&H6), 7.96–7.93 (1H, d, J = 15.0 Hz, -CH$\beta$), 8.10–8.08 (2H, d, H2’&H6’). $^{13}$C-NMR (500 MHz, DMSO) $\delta$: 186.88, 153.32, 142.44, 134.65, 130.22, 130.11, 128.86, 128.63, 127.59, 122.50, 112.47, 65.65, 46.82. ESI-MS ($m/z$): Calculated- 309.3590, Observed-309.3592.

(2E)-3-[4-methoxyphenyl]-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO3): $^1$H NMR (500 MHz, DMSO) $\delta$: 3.33–3.32 (4H, t, J = 5.0 Hz, morpholine -N-(CH$_2$)$_2$), 3.75–3.73 (4H, t, J = 5.0 Hz, morpholine O-(CH$_2$)$_2$), 3.82 (3H, s, OCH$_3$), 7.03–7.02 (2H, d, H3’ & H5’), 7.01–7.00 (2H, m, H3, & H5), 7.66–7.63 (1H, d, J = 15.0 Hz, -CH$\alpha$), 7.81–7.78 (1H, d, J = 15.0 Hz, -CH$\beta$), 7.84–7.82 (2H, d, H2&H6), 8.07–8.05 (2H, d, H2’&H6’). $^{13}$C-NMR (500 MHz, DMSO) $\delta$: 186.38, 160.89,
ESI-MS (m/z): Calculated- 323.3856, Observed-323.3853.

(2E)-3-(4-methylphenyl)-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO4): $^1$H NMR (500 MHz, DMSO) $\delta$: 2.35 (3H, s, OCH$_3$), 3.33–3.32 (4H, t, J = 5.0 Hz, morpholine -N-(CH$_2$)$_2$), 3.75–3.74 (4H, t, J = 5.0 Hz, morpholine O-(CH$_2$)$_2$), 7.04–7.02 (2H, d, H3’ & H5’), 7.27–7.25 (2H, m, H3 & H5), 7.65–7.62 (1H, d, J = 15.0 Hz, -CH$\alpha$), 7.77–7.75 (2H, d, H2&H6), 7.88–7.85 (1H, d, J = 15.0 Hz, -CH$\beta$), 8.08–8.06 (2H, d, H2’&H6’).
$^{13}$C-NMR (500 MHz, DMSO) $\delta$: 186.46, 153.93, 142.20, 140.06, 132.17, 130.38, 129.38, 128.58, 127.50, 121.03, 112.97, 65.75, 46.64, 20.96.
ESI-MS (m/z): Calculated- 307.3862, Observed-323.3860.

(2E)-3-(4-dimethylamino)phenyl)-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO5): $^1$H NMR (500 MHz, DMSO) $\delta$: 2.99 (6H, s, (NCH$_3$)$_2$), 3.32–3.31 (4H, t, J = 5.0 Hz, morpholine -N-(CH$_2$)$_2$), 6.75–6.73 (2H, d, H3’ & H5’), 7.02–7.00 (2H, m, H3 & H5), 7.19–7.16 (1H, d, J = 15.0 Hz, -CH$\alpha$), 7.62–7.61 (2H, d, H2&H6), 7.68–7.65 (1H, d, J = 15.0 Hz, -CH$\beta$), 8.04–8.02 (2H, d, H2’&H6’).
$^{13}$C-NMR (500 MHz, DMSO) $\delta$: 186.26, 153.65, 151.60, 143.26, 130.30, 130.01, 128.19, 122.25, 116.24, 113.02, 111.66, 65.7, 46.76, 41.16. ESI-MS (m/z): Calculated- 336.4274, Observed-336.4272.

(2E)-3-(4-ethylphenyl)-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO6): $^1$H NMR (500 MHz, DMSO) $\delta$: 1.12-1.18 (3H, t, J = 10.0 Hz, CH$_3$), 2.67-2.65 (2H, q, J = 10.0 Hz, CH$_2$), 3.34–3.33 (4H, t, J = 5.0 Hz, morpholine -N-(CH$_2$)$_2$), 3.75–3.74 (4H, t, J = 5.0 Hz, morpholine O-(CH$_2$)$_2$), 7.04–7.02 (2H, d, H3’ & H5’), 7.30–7.28 (2H, m, H3 & H5), 7.67–7.64 (1H, d, J = 15.0 Hz, -CH$\alpha$), 7.79–7.77 (2H, d, H2&H6), 7.89–7.86 (1H, d, J = 15.0 Hz, -CH$\beta$), 8.08–8.06 (2H, d,
\(^{13}\)C-NMR (500 MHz, DMSO) \(\delta\): 186.48, 153.94, 146.41, 142.23, 132.45, 130.39, 128.68, 128.21, 127.15, 121.11, 112.98, 65.76, 46.65, 28.02, 15.27.

ESI-MS (m/z): Calculated-321.4128, Observed-321.4125.

\((2E)-1-[4-(morpholin-4-yl)phenyl]-3-(4-nitrophenyl)prop-2-en-1-one (MO7)\): \(^1\)H NMR (500 MHz, DMSO) \(\delta\): 3.35–3.34 (4H, t, \(J = 5.0\) Hz, morpholine -N-(CH\(_2\))\(_2\)), 3.76–3.75 (4H, t, \(J = 5.0\) Hz, morpholine O-(CH\(_2\))\(_2\)), 7.05–7.03 (2H, d, H3' & H5'), 7.75–7.72 (1H, d, J = 15.0 Hz, -CH\(\alpha\)), 8.11–8.09 (2H, d, H2'&H6'), 8.14–8.11 (1H, d, J = 15.0 Hz, -CH\(\beta\)), 8.16–8.14 (2H, m, H3 & H5), 8.28–8.26 (2H, d, H2&H6). \(^{13}\)C-NMR (500 MHz, DMSO) \(\delta\): 186.07, 154.18, 147.72, 141.51, 139.33, 130.72, 129.54, 126.97, 126.27, 123.79, 112.91, 65.74, 46.53.

ESI-MS (m/z): Calculated-338.3578, Observed-338.3570.

\((2E)-3-(4-chlorophenyl)-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO8)\): \(^1\)H NMR (500 MHz, DMSO) \(\delta\): 3.34–3.33 (4H, t, \(J = 5.0\) Hz, morpholine -N-(CH\(_2\))\(_2\)), 3.75–3.74 (4H, t, \(J = 5.0\) Hz, morpholine O-(CH\(_2\))\(_2\)), 7.04–7.02 (2H, d, H3' & H5'), 7.52–7.50 (2H, d, H2'&H6'), 7.66–7.63 (1H, d, J = 15.0 Hz, -CH\(\alpha\)), 7.92–7.90 (2H, d, H2&H6), 7.97–7.94 (1H, d, J = 15.0 Hz, -CH\(\beta\)), 8.09–8.07 (2H, m, H3 & H5). \(^{13}\)C-NMR (500 MHz, DMSO) \(\delta\): 186.29, 154.02, 140.65, 134.51, 133.89, 130.51, 130.26, 129.90, 128.77, 127.26, 122.88, 112.88, 65.74, 46.58.

ESI-MS (m/z): Calculated-327.8047, Observed-327.8045.

\((2E)-3-(4-bromophenyl)-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (MO9)\): \(^1\)H NMR (500 MHz, DMSO) \(\delta\): 3.34–3.33 (4H, t, \(J = 5.0\) Hz, morpholine -N-(CH\(_2\))\(_2\)), 3.75–3.74 (4H, t, \(J = 5.0\) Hz, morpholine O-(CH\(_2\))\(_2\)), 7.04–7.02 (2H, d, H3' & H5'), 7.64–7.61 (1H, d, J = 15.0 Hz, -CH\(\alpha\)), 7.66–7.64 (2H, d, H2'&H6'), 7.85–7.83 (2H, d, H2&H6), 7.98–7.95 (1H, d, J = 15.0 Hz, -CH\(\beta\)),
8.09–8.07 (2H, m, H3, H3 & H5). $^{13}$C-NMR (500 MHz, DMSO) δ: 186.28, 154.01, 140.72, 134.20, 131.67, 130.49, 130.47, 127.23, 123.34, 122.12, 112.91, 65.72, 46.55. ESI-MS (m/z):

Calculated- 372.2557, Observed-372.2554.
MO-1
1H_8scan DMSO (D:\Spectra) nmr 6
MO-1
1H_8scan DMSO (D:\Spectra) nmr 6
MO-3
1H_8scan DMSO [D:\Spectra] nmr 7
MO-3
1H_8scan DMSO (D:\Spectra) nmr 7

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Time Time
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NS
DS
SWH
FIRRES
AQ
RG
DS
DE
TE
DI 1
TDO
SPOL 50
NUCL
P0
P1
PLN1 22
F2 - Processing
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SF
WDN
SGB 0
LB
GB 0
PC

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1H_8scan DMSO (D:\Spectra) nmr 7
MO-3
C13CPD DMSO (D:\Spectra) nmr 7

BRUKER
AVANCE NEO
500 MHz NMR SPECT
SAIF, PANJAB UNIV
CHANDIGARH

Current Data Parameters
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ENTNO: 71
PROCNO: 1

F1 - Acquisition Parameters
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TE: 298.3 K
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D11: 0.03000000 se
ISO: 1
SC2: 125.7804233 MHz
NOC1: 1.3C
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F1: 10.00 us
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SC2: 500.1720007 MHz
NOC2: 1H
CPDPG[2]: 50.00 us
CPD2: 60.00 us
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FLM12: 0.94411001 W
FLM13: 0.17308000 W

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MO-6
1H_8scan DMSO (D:\Spectra) nmr 10
1H_8scan DMSO (D:\Spectra) nmr 10
MO-6
Cl3CPD DMSO {D:\Spectra} nmr 10

BRUKER
AVANCE NEO
500 MHz NMR SPECT
SAIF, PANJAB UNIV
CHANDIGARH

Current Data Parameters
NAME Dec24-2019
KMPDS 101
FREQNO 1

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Time_ 16:35 h
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TD 68936
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CS 0
SNR 3037.035 Hz
FIDRSS 1.130281 Hz
AQ 0.6867360 sec
RO 101
DM 13.500 us
DE 6.50 us
TE 298.4 K
D1 2.00000000 sec
D11 0.33000000 sec
D2 2
SFO1 128.78045333 MHz
DCO1 13C
F0 3.33 us
P1 10.00 us
FLM1 79.56079701 W
SFO2 500.1220070 MHz
DCO2 1N
CPDPRG12 waltz65
PCD2 80.00 us
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FLM12 0.94411001 W
FLM15 0.17308000 W

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SSB 0
LB 1.00 Hz
GB 0
WC 1.40
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MO-7
1H_8scan DMSO (D:\Spectra) nmr 11

BRUKER
AVANCE NEO
500 MHz NMR SPE
SAIF, PAKISTAN UNIVERSITY OF CHEMICAL SCIENCES

Current Data File
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EXPNO PROCNO

F2 - Acquisition
Date_ Time
INSTRUM Avance PROBHD 21194
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SOLVENT NS
DS SNH
FIRES AQ
RG DS
DE TE
DI 1
TD0 SPO1 50
NUC1 P0 F1
PLN1 22

F2 - Processing
SI SF
WDN SB 0
LB GB 0
PC

8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 ppm
MO-7
1H_8scan DMSO (D:\Spectra) nmr 11
C13CPD DMSO (D:\Spectra) nmr 11
C13CPD DMSO {D:\Spectra} nmr 12
MuSSel prediction of the lead molecules……………………………………………………………………S3

MuSSel prediction of the lead MO1

| Position | Target                                         | Score       | Reliability | Similar |
|----------|-----------------------------------------------|-------------|-------------|---------|
| 1        | Calpain 2: Sus scrofa                         | 91.683 %    | YES         | 1       |
| 2        | Caspase-3: Homo sapiens                       | 75.868 %    | YES         | 5       |
| 3        | Sentrin-specific protease 7: Homo sapiens    | 75.868 %    | YES         | 5       |
| 4        | Sentrin-specific protease 8: Homo sapiens    | 75.868 %    | YES         | 5       |
| 5        | Sentrin-specific protease 6: Homo sapiens    | 75.868 %    | YES         | 5       |
| 6        | Glycogen synthase kinase-3 beta: Homo sapiens| 60.719 %    | YES         | 3       |
| 7        | Arachidonate 5-lipoxygenase: Rattus norvegicus| 57.694 %    | YES         | 5       |
| 8        | Cathepsin L: Homo sapiens                     | 50.677 %    | YES         | 5       |
| 9        | Cathepsin B: Homo sapiens                     | 50.677 %    | YES         | 5       |
| 10       | DNA-dependent protein kinase: Homo sapiens    | 45.533 %    | NO          | 4       |
| 11       | Protein skinhead-1: Caenorhabditis elegans    | 44.933 %    | NO          | 7       |
| 12       | Protein-tyrosine phosphatase 1B: Homo sapiens | 40.958 %    | NO          | 5       |
| 13       | Monoamine oxidase B: Homo sapiens            | 40.916 %    | NO          | 7       |
| 14       | Acetylcholinesterase: Homo sapiens           | 39.023 %    | NO          | 7       |
| 15       | Integrase: Human immunodeficiency virus 1     | 37.149 %    | NO          | 4       |
| 16       | Heat shock protein Hsp-16.2: Caenorhabditis elegans | 34.073 %    | NO          | 1       |
| 17       | Beta Lactamase: Pseudomonas aeruginosa        | 34.060 %    | NO          | 7       |
| 18       | PI3-kinase p110-delta subunit: Homo sapiens   | 34.007 %    | NO          | 2       |
| 19       | PI3-kinase p110-gamma subunit: Homo sapiens   | 34.007 %    | NO          | 2       |
| 20       | PI3-kinase p110-beta subunit: Homo sapiens    | 34.007 %    | NO          | 2       |
**MuSSel prediction of the lead MO5**

| Position | Target 1 | Target 2       | Score   | Reliability | Similar | Score 2 | Reliability | Similar |
|----------|----------|----------------|---------|-------------|---------|---------|-------------|---------|
| 1        | Calpain 2: Sus scrofa |              | 86.747% (11.277) | YES | 1 | | |
| 2        | Senlin-specific protease 6: Homo sapiens |  | 65.432% (8.506) | YES | 5 | | |
| 3        | Senlin-specific protease 8: Homo sapiens |  | 65.432% (8.506) | YES | 5 | | |
| 4        | Caspase-3: Homo sapiens |  | 65.432% (8.506) | YES | 5 | | |
| 5        | Senlin-specific protease 7: Homo sapiens |  | 65.432% (8.506) | YES | 5 | | |
| 6        | Arachidonate 5-lipoxygenase: Rattus norvegicus |  | 53.401% (6.942) | YES | 4 | | |
| 7        | Monoamine oxidase B: Homo sapiens |  | 47.277% (6.146) | YES | 9 | | |
| 8        | Protein-tyrosine phosphatase 1B: Homo sapiens |  | 46.935% (6.102) | YES | 3 | | |
| 9        | Cathepsin L: Homo sapiens |  | 44.673% (5.807) | NO | 6 | | |
| 10       | Cathepsin B: Homo sapiens |  | 44.673% (5.807) | NO | 7 | | |
| 11       | Beta Lactamase: Pseudomonas aeruginosa |  | 43.832% (5.698) | NO | 7 | | |
| 12       | Acetylcholinesterase: Electrophorus electricus |  | 43.187% (5.614) | NO | 9 | | |
| 13       | Monoamine oxidase A: Homo sapiens |  | 42.763% (5.559) | NO | 5 | | |
| 14       | Protein skinhead-1: Caenorhabditis elegans |  | 41.656% (5.415) | NO | 6 | | |
| 15       | Acetylcholinesterase: Homo sapiens |  | 39.220% (5.099) | NO | 8 | | |
| 16       | Cyclooxygenase-2: Homo sapiens |  | 38.388% (4.990) | NO | 5 | | |
| 17       | Toll-like receptor 9: Homo sapiens |  | 37.371% (4.858) | NO | 6 | | |
| 18       | Quinolone resistance protein norA: Staphylococcus aureus |  | 35.691% (4.640) | NO | 3 | | |
| 19       | Glycogen synthase kinase-3 beta: Homo sapiens |  | 34.780% (4.521) | NO | 3 | | |
| 20       | Cholinesterase: Equus caballus |  | 34.515% (4.487) | NO | 8 | | |