Supplentary Materials for

Steroids as the novel class of high-affinity allosteric modulators of muscarinic receptors

Eva Dolejší1, Eszter Szánti-Pintér2, Nikolai Chetverikov1, Dominik Nelic1, Alena Randáková1, Vladimír Doležal1, Eva Kudová2\*, Jan Jakubík1\*

1 Institute of Physiology Czech Academy of Sciences, Prague, Czech Republic.

2, Institute of Organic Chemistry and Biochemistry Czech Academy of Sciences, Prague, Czech Republic.

\* Correspondence to: Jan Jakubik (jan.jakubik@fgu.cas.cz) and Eva Kudová (eva.kudova@uochb.cas.cz)

**This PDF file includes:**

Tables S1 to S3

# Table S1 Effect of 10 µM tested the compound on [3H]NMS binding

Effect of a tested compound at 10 μM concentration on the binding of 100 pM [3H]NMS is expressed as a per cent of [3H]NMS specific binding in the absence of the tested compound. Values are means ± SD from 3 independent experiments performed in quadruplicates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | M1 | M2 | M3 | M4 | M5 |
| 11-Deoxycorticosterone | 71 ± 4 | 115 ± 5 | 121 ± 5 | 70 ± 4 | 85 ± 6 |
| Corticosterone | 132 ± 6 | 118 ± 5 | 123 ± 5 | 123 ± 5 | 71 ± 4 |
| 5α-  Dihydrodeoxycorticosterone | 71 ± 4 | 69 ± 4 | 66 ± 4 | 121 ± 5 | 71 ± 4 |
| 17α-Hydroxyprogesterone | 71 ± 4 | 88 ± 5 | 85 ± 5 | 81 ± 4 | 76 ± 4 |
| Pregnenolone | 124 ± 5 | 62 ± 4 | 48 ± 3 | 127 ± 6 | 67 ± 4 |
| Progesterone | 124 ± 5 | 112 ± 5 | 130 ± 6 | 116 ± 5 | 124 ± 5 |
| 5β-Dihydroprogesterone | 75 ± 4 | 87 ± 4 | 71 ± 4 | 134 ± 6 | 70 ± 4 |
| 5α-Dihydroprogesterone | 51 ± 3 | 56 ± 3 | 56 ± 4 | 55 ± 3 | 64 ± 4 |
| Epipregnanolone | 71 ± 4 | 59 ± 4 | 55 ± 3 | 70 ± 4 | 86 ± 4 |
| Pregnanolone | 82 ± 4 | 153 ± 8 | 63 ± 4 | 63 ± 4 | 83 ± 4 |
| Isopregnanolone | 70 ± 4 | 66 ± 4 | 68 ± 4 | 134 ± 6 | 71 ± 4 |
| Allopregnanolone | 66 ± 4 | 45 ± 3 | 54 ± 3 | 43 ± 3 | 61 ± 4 |
| Estradiol | 62 ± 4 | 70 ± 4 | 73 ± 4 | 92 ± 5 | 63 ± 4 |
| DHEA | 71 ± 4 | 119 ± 5 | 72 ± 4 | 112 ± 5 | 66 ± 4 |
| Androstenediol | 64 ± 4 | 65 ± 4 | 74 ± 4 | 71 ± 4 | 67 ± 4 |
| Androstenedione | 47 ± 3 | 84 ± 4 | 126 ± 6 | 73 ± 4 | 46 ± 3 |
| Testosterone | 132 ± 7 | 120 ± 5 | 54 ± 3 | 70 ± 4 | 69 ± 4 |
| Dihydrotestosterone | 71 ± 4 | 60 ± 4 | 49 ± 3 | 71 ± 4 | 56 ± 3 |
| 5α-androstenedione | 73 ± 4 | 71 ± 4 | 72 ± 4 | 74 ± 4 | 71 ± 4 |
| 5β-androstenedione | 73 ± 4 | 69 ± 4 | 66 ± 4 | 41 ± 3 | 72 ± 4 |

# Table S2 Factors of binding cooperativity

Factors of binding cooperativity α between tested compounds and [3H]NMS were obtained by fitting equation Eq. 2 to data from equilibrium binding experiments. Values are means ± SD from 3 independent experiments performed in quadruplicates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | M1 | M2 | M3 | M4 | M5 |
| 11-Deoxycorticosterone | n.d. | n.d. | 1.29 ± 0.07 | n.d. | n.d. |
| Corticosterone | 1.96 ± 0.38 | 1.18 ± 0.06 | 1.30 ± 0.13 | 1.30 ± 0.15 | n.d. |
| 5α-  Dihydrodeoxycorticosterone | n.d. | n.d. | n.d. | 0.80 ± 0.08 | n.d. |
| 17α-Hydroxyprogesterone | n.d. | 0.78 ± 0.05 | 0.72 ± 0.07 | n.d. | 0.61 ± 0.04 |
| Pregnenolone | 1.33 ± 0.09 | n.d. | n.d. | 1.41 ± 0.16 | n.d. |
| Progesterone | 1.33 ± 0.16 | 1.28 ± 0.15 | 1.49 ± 0.07 | 1.14 ± 0.06 | 1.32 ± 0.10 |
| 5β-Dihydroprogesterone | 0.56 ± 0.04 | 0.77 ± 0.05 | n.d. | 1.64 ± 0.13 | 0.50 ± 0.04 |
| 5α-Dihydroprogesterone | n.d. | n.d. | n.d. | n.d. | n.d. |
| Epipregnanolone | n.d. | n.d. | n.d. | 0.53 ± 0.08 | 0.74 ± 0.02 |
| Pregnanolone | 0.67 ± 0.05 | 3.33 ± 0.44 | n.d. | 0.44 ± 0.12 | 0.71 ± 0.04 |
| Isopregnanolone | n.d. | n.d. | n.d. | 1.69 ± 0.11 | n.d. |
| Allopregnanolone | n.d. | n.d. | n.d. | n.d. | n.d. |
| Estradiol | n.d. | n.d. | n.d. | 0.84 ± 0.03 | n.d. |
| DHEA | n.d. | 1.20 ± 0.06 | n.d. | 1.30 ± 0.08 | n.d. |
| Androstenediol | n.d. | n.d. | n.d. | n.d. | n.d. |
| Androstenedione | n.d. | 0.72 ± 0.03 | 1.37 ± 0.08 | n.d. | n.d. |
| Testosterone | 1.59 ± 0.15 | 1.22 ± 0.07 | n.d. | n.d. | n.d. |
| Dihydrotestosterone | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5α-androstenedione | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5β-androstenedione | n.d. | n.d. | n.d. | n.d. | n.d. |

# Table S3 Parameters of functional response to acetylcholine

Maximal response of the system EMAX and parameters of functional response to acetylcholine half-efficient concentration of acetylcholine EC50, maximal response to acetlylcholine E’MAX were obtained by fitting Eq. 4 to concentration response curves. Operational efficacy of acetylcholine τ was calculated according to Eq. 5. Values of EC50 are expressed as negative logarithms. Values are means ± SD from 3 independent experiments performed in quadruplicates.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | EMAX | pEC50 | E’MAX | τ |
| M1 | 6.5 ± 0.5 | 6.88 ± 0.06 | 3.29 ± 0.07 | 0.713 ± 0.030 |
| M2 | 8.5 ± 0.7 | 6.67 ± 0.07 | 4.23 ± 0.14 | 0.756 ± 0.050 |
| M3 | 6.6 ± 0.5 | 6.57 ± 0.06 | 3.12 ± 0.08 | 0.609 ± 0.031 |
| M4 | 8.2 ± 0.7 | 6.43 ± 0.05 | 3.51 ± 0.09 | 0.535 ± 0.027 |
| M5 | 6.3 ± 0.5 | 6.63 ± 0.06 | 2.87 ± 0.07 | 0.545 ± 0.027 |