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  |