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Positive Modulators of the *N*-Methyl-D-Aspartate Receptor: Structure-Activity Relationship Study on Steroidal 3-Hemiesters

Barbora Krausova[1], Barbora Slavikova[2], Michaela Nekardova[1,3], Pavla Hubalkova[1,4], Vojtech Vyklicky[1], Hana Chodounska[2], Ladislav Vyklicky[1], Eva Kudova[2,]*

[1]Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, Prague 4, 142 20, Czech Republic

[2]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 2, Prague 6 – Dejvice, 166 10, Czech Republic

[3]Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 3, Prague 2, 121 16, Czech Republic

[4] Third Faculty of Medicine, Charles University in Prague, Ruska 87, Prague 10, 100 00, Czech Republic

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ABSTRACT. Here, we report the synthesis of pregn-5-ene and androst-5-ene dicarboxylic acid esters and explore the structure-activity relationship (SAR) for their modulation of *N*-methyl-*D*-aspartate receptors (NMDARs). All compounds were positive modulators of recombinant GluN1/GluN2B receptors (EC_{50} varying from 1.8 to 151.4 μM and E_{max} varying from 48 to 452 %). Moreover, 10 compounds were found to be more potent GluN1/GluN2B receptor modulators than endogenous pregnenolone sulfate (EC_{50} = 21.7 μM). The SAR study revealed a relationship between the length of the residues at carbon C-3 of the steroid molecule and the positive modulatory effect at GluN1/GluN2B receptors for various D-ring modifications. A selected compound – 20-oxo-pregnenolone hemiadipate – potentiated native NMDARs to a similar extent as GluN1/GluN2A-D receptors, and inhibited AMPARs and $GABA_AR$ responses. These results provide a unique opportunity for the development of new steroid-based drugs with potential use in the treatment of neuropsychiatric disorders involving hypofunction of NMDARs.

Introduction.

N-methyl-*D*-aspartate receptors (NMDARs) are glutamate-gated, calcium-permeable ion channels that are activated during excitatory synaptic transmission and are implicated in various forms of synaptic plasticity,[1] which underlies learning and memory processes.[2] Next-generation genome sequencing allowed identification of mutations in *GRIN* genes encoding for human NMDAR subunits that have been associated with various neurodevelopmental disorders.[3] The analysis of mutated receptors revealed various forms of trafficking and functional defects.[4] Positive allosteric modulators (PAMs) that increase the activity of NMDARs may provide a therapeutic aid for patients suffering from neuropsychiatric disorders where NMDARs hypofunction is thought to be involved, such as intellectual disability,[5] autism spectrum disorder[6] or schizophrenia.[7]

Neurosteroids are endogenous steroidal compounds that modulate the activity of NMDARs.[8] Despite structural similarity between different neurosteroids, they can exhibit opposite effects at NMDARs, apparently mediated by different molecular mechanisms. The inhibitory effect of neurosteroids on NMDARs is dependent upon a 3α-negatively charged substituent and 5β-stereochemistry.[9] This class of neurosteroids is represented by endogenous pregnanolone sulfate (20-oxo-5β-pregnan-3α-yl sulfate, **Figure 1A**).[10] In contrast, a 3β-negatively charged moiety in combination with Δ^5- stereochemistry (a double bond between C-5 and C-6) favors potentiation of NMDARs.[9a,11] This class of neurosteroids is represented by endogenous pregnenolone sulfate (20-oxo-pregn-5-en-3β-yl sulfate, compound **1**, **PES**, **Figure 1B**).[12] **PES** is an abundantly occurring neurosteroid synthesized *de novo* in the central nervous system, which exhibits different modulatory effects on several types of receptors: specifically potentiating the responses elicited by NMDARs[12] while inhibiting currents mediated by γ-aminobutyric acid type A

receptors ($GABA_ARs$),[13] glycine receptors[14] and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs).[12] A memory enhancement effect after the administration of **PES** *in vivo* has been reported.[15]

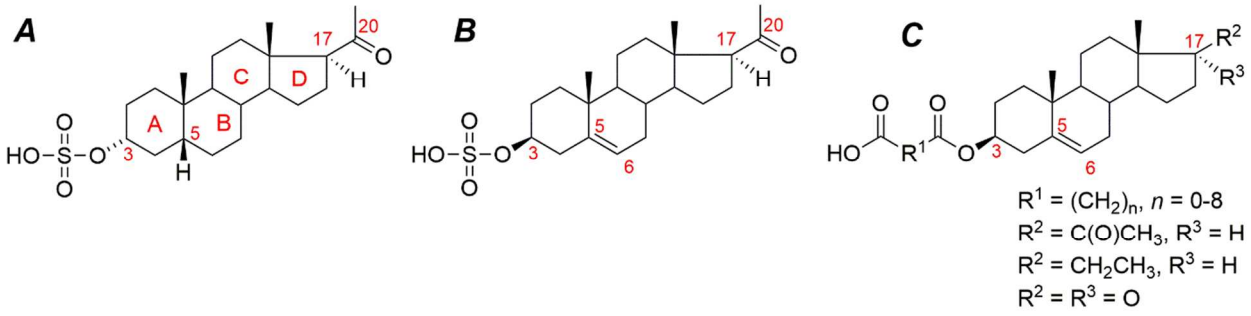


Figure 1. Structure of *(A)* pregnanolone sulfate, *(B)* pregnenolone sulfate (**1**, **PES**) and *(C)* hemiester analogues of **PES**.

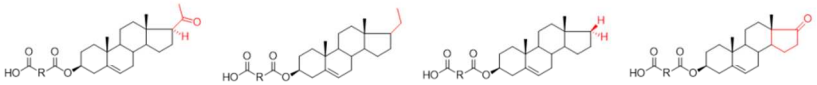
A structurally distinct class of compounds that act as PAMs of NMDARs, represented by (3-chlorophenyl)(6,7-dimethoxy-1-((4-methoxyphenoxy)methyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (CIQ), was identified by an extensive study involving 100,000 substances focused on selective action at NMDARs containing GluN2C or GluN2D subunits.[16] The most effective CIQ analogs have EC_{50} values in the submicromolar range and induce an approximately two-fold potentiation of GluN1/GluN2D receptor responses.[17] By replacing the dimethyl groups on the C-ring with one isopropoxyl group, CIQ analogs can be targeted to the GluN2B subunit.[18] However, the analogue that acts solely at GluN2B subunits exhibits only moderate efficacy and potency (E_{max} = 152%; EC_{50} = 7.2 μM).[18]

The SAR for steroid-based PAMs of NMDARs is only poorly understood and therefore needs to by further characterized, especially in the context of the development of new drug candidates for the prevention and/or treatment of neuropsychiatric disorders and age-related memory

deficits.[4,19] Here, we describe a SAR study on hemiester analogues **2-24** (**Figure 1C** and **Table 1**) with varying lengths of C-3 substituents and different steroidal D-ring modifications, primarily focusing on fine-tuning their modulatory effect on NMDARs. The biological activity of compounds **2-24** was evaluated on recombinant GluN1/GluN2B receptors expressed in human embryonic kidney (HEK293) cells. The effect of a selected compound, 20-oxo-pregn-5-ene hemiadipate (**6**), was further analyzed on recombinant GluN1/GluN2A-D receptors, and native NMDARs, AMPARs, and $GABA_A$Rs expressed in hippocampal neurons.

Results and Discussion

Table 1. Structures of **PES** analogues − compounds **2-24**.

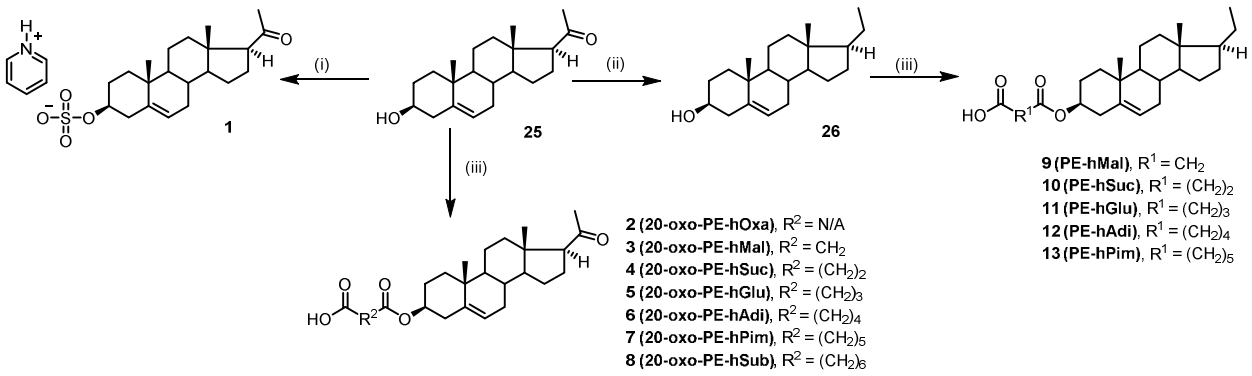


C-3 Moiety \ Steroid	20-Oxo-pregn-5-ene compounds	Pregn-5-ene compounds	Androst-5-ene compounds	17-Oxo-androst-5-ene compounds
C_2 (hemiOxalate), R = none	2 (20-Oxo-PE-hOxa)	-	-	-
C_3 (hemiMalonate), R = CH_2	3 (20-Oxo-PE-hMal)	9 (PE-hMal)	14 (AND-hMal)	-
C_4 (hemiSuccinate), R = $(CH_2)_2$	4 (20-Oxo-PE-hSuc)	10 (PE-hSuc)	15 (AND-hSuc)	-
C_5 (hemiGlutarate), R = $(CH_2)_3$	5 (20-Oxo-PE-hGlu)	11 (PE-hGlu)	16 (AND-hGlu)	19 (17-Oxo-AND-hGlu)
C_6 (hemiAdipate), R = $(CH_2)_4$	6 (20-Oxo-PE-hAdi)	12 (PE-hAdi)	17 (AND-hAdi)	20 (17-Oxo-AND-hAdi)
C_7 (hemiPimelate), R = $(CH_2)_5$	7 (20-Oxo-PE-hPim)	13 (PE-hPim)	18 (AND-hPim)	21 (17-Oxo-AND-hPim)
C_8 (hemiSuberate), R = $(CH_2)_6$	8 (20-Oxo-PE-hSub)	-	-	22 (17-Oxo-AND-hSub)
C_9 (hemiAzelate), R = $(CH_2)_7$	-	-	-	23 (17-Oxo-AND-hAze)
C_{10} (hemiSebacate), R = $(CH_2)_8$	-	-	-	24 (17-Oxo-AND-hSeb)

Chemistry.

The synthesis of compounds **1-13** is summarized in **Scheme 1**. Compounds **1-8** were prepared from commercially available 20-oxo-pregn-5-en-3β-ol (pregnenolone, compound **25**, Steraloids, Newport, RI, USA). Compounds **9-13** were prepared from 3β-hydroxy-pregn-5-ene (**26**), which was obtained by the Zn/TMSCl-mediated Clemmensen reduction[20] of pregnenolone **25** (78% yield). Pyridinium salt of pregnenolone sulfate (**1**) was prepared by the treatment of pregnenolone (**25**) with a sulfur trioxide-pyridine complex in $CHCl_3$ (89% yield). Note that compound **1** is not commercially available as is sodium salt and in our experiments exerts better solubility and chemical stability. Compound **2** was prepared by the treatment of pregnenolone **25**

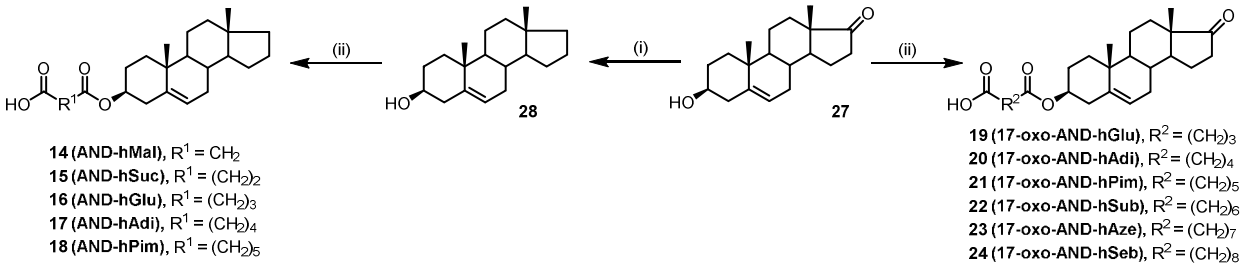
with oxalyl chloride in DCM, Et_3N and DMF (36% yield). Compounds **3** and **9** were prepared by the treatment of **25** and **26**, respectively, with Meldrum´s acid in toluene at 80 °C (37% and 32% yield, respectively). Compounds **4**, **10** and **5**, **11** were prepared by the treatment of **25** and **26** respectively with succinic or glutaric anhydride and DMAP in pyridine at 110 °C, yielding hemiesters **4** (52% yield), **5** (51% yield), **10** (43% yield), and **11** (44% yield). Compounds **6**-**8** and **12**, **13** were prepared by the treatment of **25** and **26,** respectively with adipic, pimelic, or suberic acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), N,N-diisopropylethylamine (DIPEA) and DMAP in DCM, yielding hemiesters **6** (42% yield), **7** (47% yield), **8** (44% yield), **12** (21% yield), and **13** (35% yield).



Scheme 1. Synthesis of pregn-5-ene derivatives (**1**-**13**)[a]

[a]Reagents and conditions: *(i)* sulfur trioxide-pyridine complex, $CHCl_3$, pyridine; *(ii)* Zn, TMSCl, MeOH, DCM; *(iii)* for compound **2**: oxalyl chloride, Et_3N, DCM, DMF; for compound **3** and **9**: Meldrum´s acid, toluene, 80°C; for compound **4** and **10**: succinic anhydride, DMAP, pyridine, 110 °C; for compound **5** and **11**: glutaric anhydride, DMAP, pyridine, 110 °C; for compounds **6** and **12**: adipic acid, EDCI, DIPEA, DMAP, DCM; for compound **7** and **13**: pimelic acid, EDCI, DIPEA, DMAP, DCM; for compound **8**: suberic acid, EDCI, DIPEA, DMAP, DCM.

The synthesis of compounds **14-24** is summarized in **Scheme 2**. Compounds **19-24** were prepared from commercially available 17-oxo-androst-5-en-3β-ol (**27**, Steraloids, Newport, RI, USA). Compounds **14-18** were prepared from 3β-hydroxy-androst-5-ene (**28**),[21] which was obtained by the Zn/TMSCl-mediated Clemmensen reduction[20] of compound **27** (75% yield). Compound **14** was prepared by the treatment of **28** with Meldrum´s acid in toluene at 80 °C (13% yield). Compounds **15**, **16** and **19** were prepared by the treatment of **27** and **28** respectively with succinic or glutaric anhydride and DMAP in pyridine at 110 °C, yielding hemiesters **15** (67% yield), **16** (42% yield), and **19** (58% yield). Compounds **17**, **18** and **20-24** were prepared by the treatment of **27** and **28**, respectively with adipic, pimelic, suberic, azelaic, or sebacic acid, EDCI, DIPEA and DMAP in DCM, yielding hemiesters **17** (35% yield), **18** (42% yield), **20** (31% yield), **21** (35% yield), **22** (50% yield), **23** (24% yield), and **24** (27% yield). Although these unoptimized reactions provided low yields of the desired products, we have demonstrated the feasibility of this approach to obtain compounds **14-24**.



Scheme 2. Synthesis of androst-5-ene derivatives (**14-24**)[a]

[a]Reagents and conditions: *(i)* Zn, TMSCl, MeOH, DCM; *(ii)* for compound **14**: Meldrum´s acid, toluene, 80 °C; for compound **15**: succinic anhydride, DMAP, pyridine, 110 °C; for compound **16** and **19**: glutaric anhydride, DMAP, pyridine, 110 °C; for compound **17** and **20**: adipic acid, EDCI, DIPEA, DMAP, DCM; for compound **18** and **21**: pimelic acid, EDCI,

DIPEA, DMAP, DCM; for compound **22**: suberic acid, EDCI, DIPEA, DMAP, DCM; for compound **23**: azelaic acid, EDCI, DIPEA, DMAP, DCM; for compound **24**: sebacic acid, EDCI, DIPEA, DMAP, DCM.

Biological Activity.

To investigate the effect of **PES** (**1**) and its analogues (compounds **2**-**24**; **Figure 1C** and **Table 1**) on the activity of NMDARs, electrophysiological measurements were performed on HEK293 cells that were co-transfected with cDNAs containing genes encoding for the rat GluN1-1a and GluN2B subunits. The degree of modulation (*E;* potentiation/inhibition) for the newly synthesized compounds was determined using the following formula:

$$E = \frac{(I_e - I_a)}{I_a} \times 100 \qquad \text{Equation (1),}$$

where I_e is the value of the current amplitude during glutamate and compound co-application and I_a is the current amplitude value for glutamate application. The degree of potentiation (%) was determined for five compound doses, differing 100-fold in their concentration range, in individual cells, and data were fit to the following equation:

$$E = E_{max} / \left(1 + \left(\frac{EC_{50}}{[compound]}\right)^h\right) \qquad \text{Equation (2),}$$

where E_{max} is the maximal value of potentiation induced by a saturating concentration of the compound, EC_{50} is the concentration of the compound that produces half-maximal potentiation of the agonist-evoked current, [*compound*] is the compound concentration and *h* is the apparent Hill coefficient.

The results are presented as mean ± SEM, with *n* equal to the number of studied cells. Unpaired Student's t-test was used to perform a statistical comparison between two treatment groups and One-way Analysis of Variance (ANOVA) for multiple comparisons (unless otherwise stated, a value of $P \leq 0.05$ was used for the determination of significance).

Compounds 2-24 Potentiate Recombinant GluN1/GluN2B Receptor Responses.

We have evaluated a relationship between the steroid structure and its modulatory effect on GluN1/GluN2B receptors for compounds **2-24**. **Figure 2** shows that compound **6** (30 μM) potentiates GluN1/GluN2B receptor responses. The degree of potentiation was affected by the relative timing of compound and glutamate application and dependent on glutamate concentration. The responses induced by 1 μM glutamate were potentiated by 136 ± 8% (n = 10) when compound **6** was co-applied with the agonist. The potentiation was only 75 ± 1% (n = 6) when compound **6** was co-applied with a saturating concentration of glutamate (1 mM). The responses to 1 mM glutamate were potentiated by 134 ± 7% (n = 8) when compound **6** was pre-applied prior to glutamate (not significantly different from the potentiation induced at a low concentration of glutamate; unpaired Student's t-test, P = 0.398). These results are in agreement with the disuse-dependent effect of **PES** at NMDARs.[22] In subsequent experiments we used compound co-aplication with 1 μM glutamate.

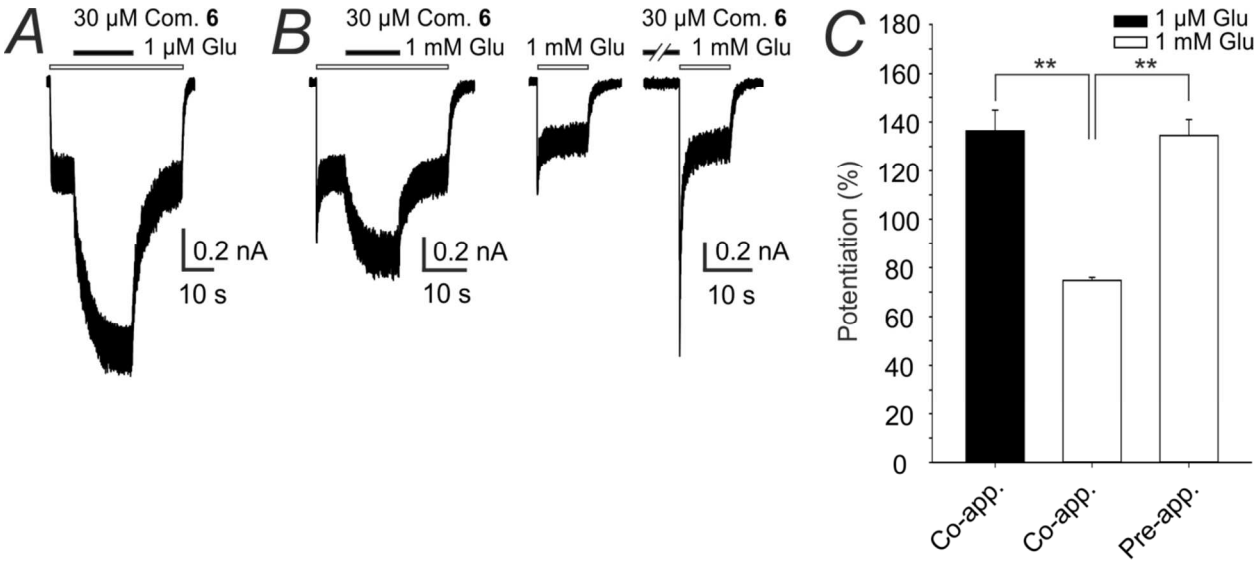


Figure 2. The potentiating effect of compound **6** at GluN1/GluN2B receptors. Examples of traces obtained from HEK293 cells transiently expressing GluN1/GluN2B receptors. *(A)* Compound **6** (30 μM) was applied simultaneously with 1 μM glutamate (the duration of compound **6** and glutamate application is indicated by filled and open bars, respectively). *(B)* Compound **6** (30 μM) was either co-applied with 1 mM glutamate (on the left), or pre-applied before the receptor was activated by 1 mM glutamate (control and potentiated response on the right). *(D)* Bar graph shows the degree of potentiation by compound **6** (30 μM) when co-applied with 1 μM glutamate (filled bar), co-applied with 1 mM glutamate (open bar) and pre-applied prior to 1 mM glutamate (open bar). Asterisks mark significance at the level of $P \leq 0.001$.

In initial experiments, we analyzed the effect of compounds **2-24** at a concentration of 30 μM on GluN1/GluN2B receptors. **Figure 3** shows the SAR for the series of 20-oxo-pregn-5-enes (**2-8**), pregn-5-enes (**9-13**), androst-5-enes (**14-18**), and 17-oxo-androst-5-enes (**19-24**). The analysis of the potentiating effect indicates that there is an optimal length of the substituents at C-3 in combination with structural modification at C-17 of the steroidal D-ring for acquiring the

desired biological effect. Once the 20-oxo substituent of 20-oxo-PE-hAdi (**6**) was removed, the most efficacious PAM was PE-hGlu (**11**), which has a C-3 moiety with five carbons. Analogously, when the steroidal skeleton does not bear the C-17 acyl, the most efficacious PAM was AND-hSuc (**15**), which has a C-3 moiety with four carbons. Such common structural features for compounds **2**-**24** may characterize the pharmacophore of steroidal structural requirements for the optimal potentiating effect at NMDARs. Previously, compounds **2**, **4**, and **5** were found to enhance NMDARs-induced Ca^{2+} accumulation in cultured hippocampal neurons.[9a]

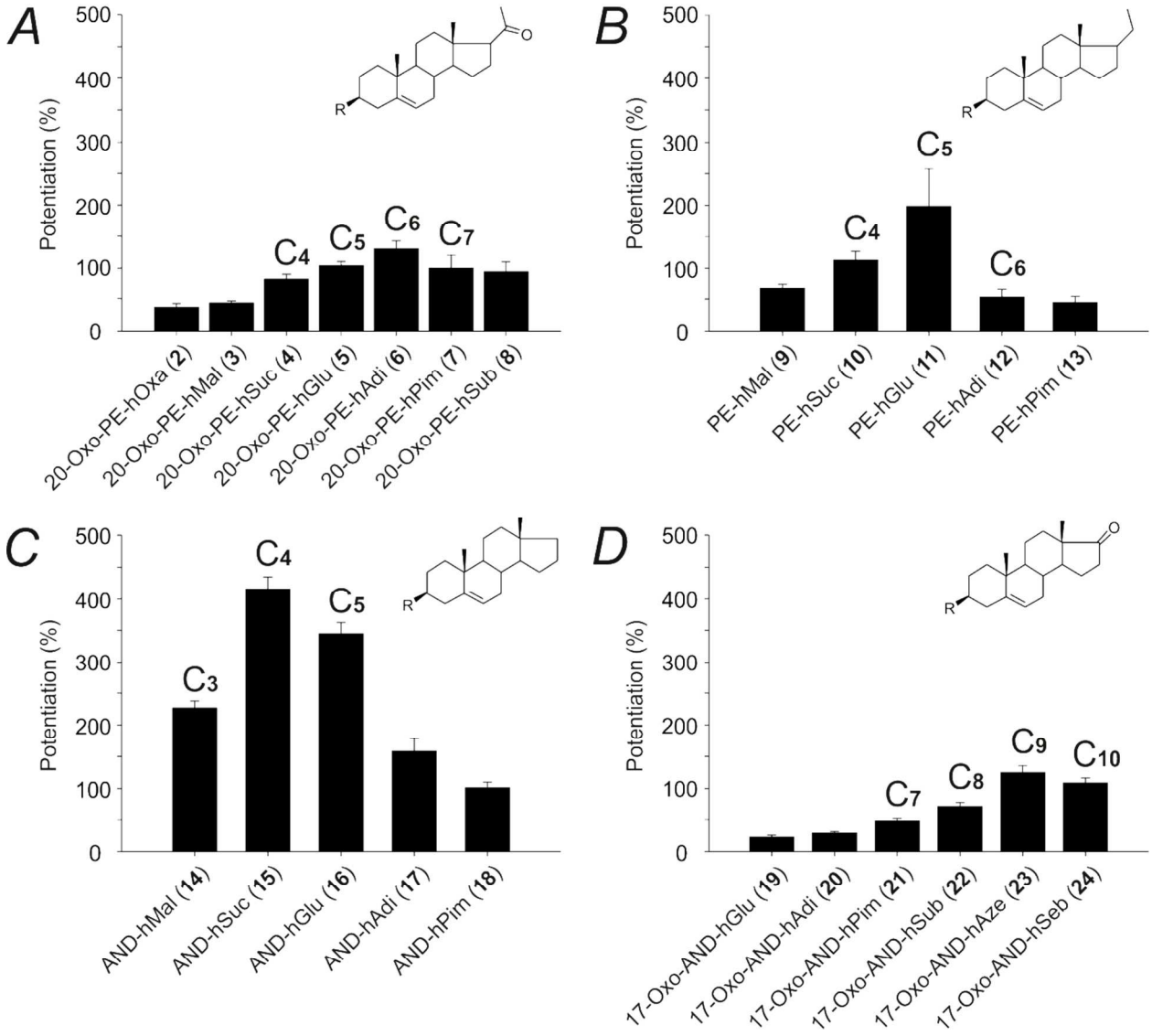


Figure 3. The effect of compounds **2-24** on current responses of GluN1/GluN2B receptors. Bar graphs show changes in response to the application of compounds **2-24** at a concentration of 30 μM. The numbers above the bars indicate the number of carbons of the particular hemiester moiety. (*A*) 20-Oxo-pregn-5-en-3β-yl hemiesters. (*B*) Pregn-5-en-3β-yl hemiesters. (*C*) Androst-5-en-3β-yl hemiesters. (*D*) 17-Oxo-androst-5-en-3β-yl hemiesters. Differences in mean values within the structurally related groups (**2-8**, **9-13**, **14-18** and **19-24**) were statistically significant (One Way ANOVA; $P < 0.001$).

Next, we have performed dose-response analysis for the effect of compounds **1-24** on GluN1/GluN2B receptor responses induced by 1 μM glutamate (**Figure 4**). Fit to the Equation (2) provided information on the compounds **1-24** efficacy and potency. The E_{max} and EC_{50} values for compounds **1-24** are shown in **Table 2**. Compounds **1-8** and **21-24** were measured up to a concentration of 100 μM and compounds **19** and **20** up to a concentration of 300 μM. Compounds **9-18** were measured up to a concentration of 30 μM due to their limited solubility. Our data show that 17 out of 23 newly synthesized compounds had increased efficacy and/or potency at GluN1/GluN2B receptors as compared to endogenous **PES (1)**.

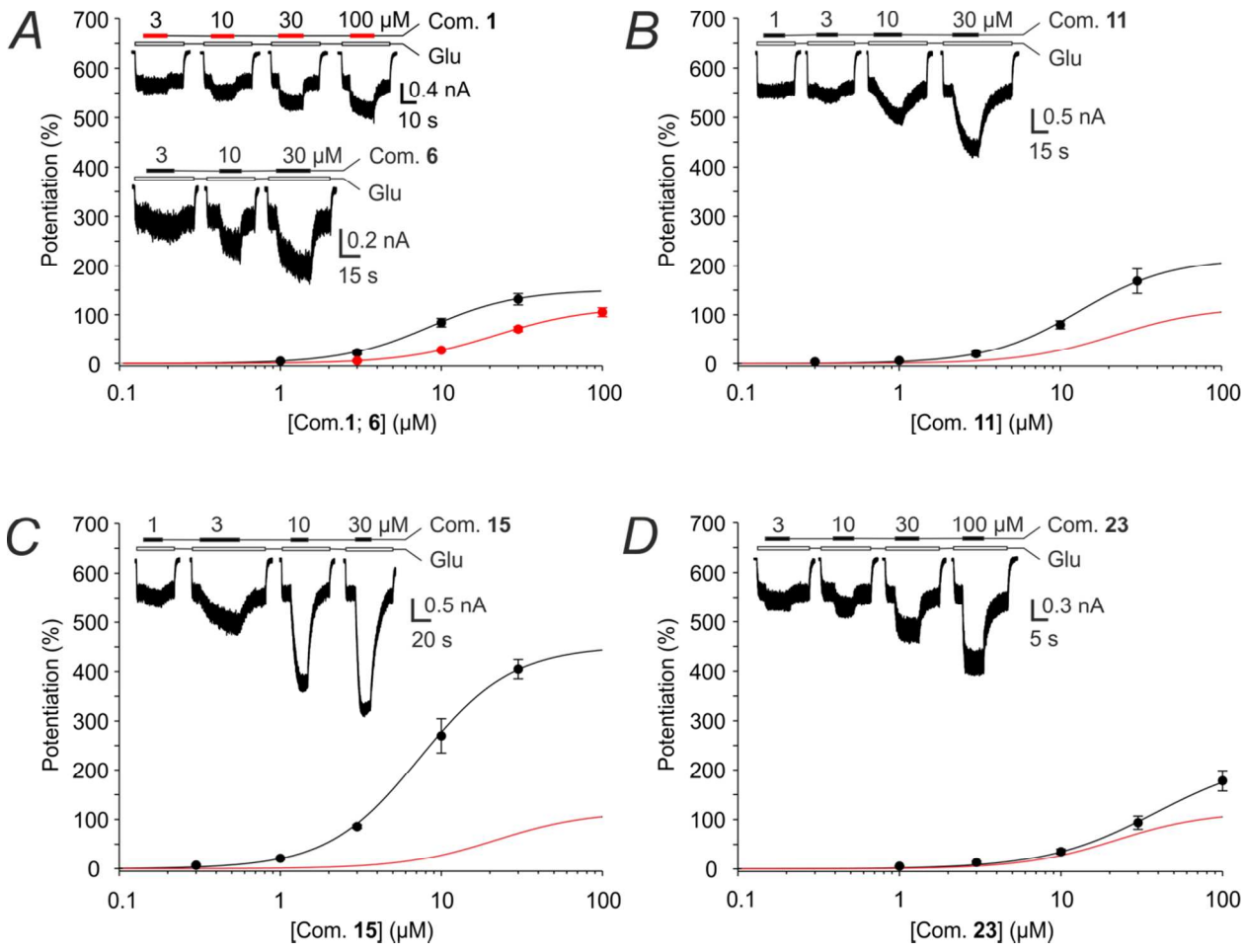


Figure 4. The effect of compounds **1**, **6**, **11**, **15** and **23** on GluN1/GluN2B receptors. Graphs show the dose-response curves for the effect of the following compounds: (*A*) **PES** (**1**; in red) and 20-Oxo-PE-hAdi (**6**), (*B*) PE-hGlu (**11**), (*C*) AND-hSuc (**15**) and (*D*) 17-Oxo-AND-hAze (**23**) at GluN1/GluN2B receptors. The red line in (*B-D*) repeats the fitted function for **PES (1)**, for comparison. Data points are averaged potentiation values from at least four independent measurements. Error bars represent SEM. The degree of potentiation of glutamate-induced responses recorded in the presence of compound **1** (3-100 μM), **6** (1-30 μM), **11** (0.3-30 μM), **15** (0.3-30 μM) and **23** (1-100 μM) was determined in individual cells and data were fitted to the Equation (2). Insets show examples of traces obtained from HEK293 cells co-transfected with cDNAs encoding for GluN1 and GluN2B subunits. Compounds **1**, **6**, **11**, **15** and **23** were applied simultaneously with 1 μM glutamate (duration of compound and glutamate application is indicated by filled and open bars, respectively, **PES** (**1**) in red).

Table 2. Summary of the dose-response analysis data for the potentiating effect of compounds **1**-**24** at GluN1/GluN2B receptor responses.

Steroid	E_{max} ± SEM (%)	EC_{50} ± SEM (μM)	*h* ± SEM	*n*
PES (1)	116 ± 10	21.7 ± 1.6	1.5 ± 0.1	8
20-Oxo-PE-hOxa (2)	93 ± 25	48.6 ± 12.4*	1.3 ± 0.0	5
20-Oxo-PE-hMal (3)	108 ± 15	39.8 ± 10.3	1.3 ± 0.1	5
20-Oxo-PE-hSuc (4)	177 ± 46*	27.6 ± 12.1	1.5 ± 0.2	4
20-Oxo-PE-hGlu (5)	183 ± 25*	19.0 ± 3.4	1.4 ± 0.1	4
20-Oxo-PE-hAdi (6)	151 ± 16	8.5 ± 1.0**	1.6 ± 0.1	10
20-Oxo-PE-hPim (7)	191 ± 63	18.0 ± 7.9	1.4 ± 0.2	5

20-Oxo-PE-hSub (8)	156 ± 30	19.3 ± 1.7	1.5 ± 0.1	5
One-way ANOVA	P = 0.346	**P < 0.001**	P = 0.494	
PE-hMal (9)	71 ± 7*	10.7 ± 0.8**	1.5 ± 0.2	7
PE-hSuc (10)	133 ± 19	9.9 ± 5.2*	1.4 ± 0.2	6
PE-hGlu (11)	209 ± 22*	9.9 ± 3.7*	1.8 ± 0.3	5
PE-hAdi (12)	60 ± 12*	17.2 ± 4.5	1.5 ± 0.4	4
PE-hPim (13)	48 ± 11*	26.2 ± 8.1	1.4 ± 0.4	4
One-way ANOVA	**P = 0.002**	P = 0.080	P = 0.820	
AND-hMal (14)	236 ± 27**	4.7 ± 1.0**	1.4 ± 0.2	4
AND-hSuc (15)	452 ± 46*	7.4 ± 0.4**	1.5 ± 0.1	5
AND-hGlu (16)	348 ± 32**	5.2 ± 1.2**	1.7 ± 0.2	7
AND-hAdi (17)	191 ± 10**	2.8 ± 0.5**	1.7 ± 0.5	5
AND-hPim (18)	109 ± 13	1.8 ± 0.3**	1.8 ± 0.3	4
One-way ANOVA	**P <0.001**	**P < 0.001**	P = 0.892	
17-Oxo-AND-hGlu (19)	154 ± 34	151.4 ± 32.7**	1.2 ± 0.1	4
17-Oxo-AND-hAdi (20)	180 ± 17*	108.9 ± 20.2**	1.4 ± 0.1	7
17-Oxo-AND-hPim (21)	187 ± 27*	82.4 ± 16.9**	1.2 ± 0.1	7
17-Oxo-AND-hSub (22)	189 ± 35*	49.1 ± 13.7**	1.4 ± 0.2	5
17-Oxo-AND-hAze (23)	226 ± 45*	38.2 ± 14.5	1.3 ± 0.2	5
17-Oxo-AND-hSeb (24)	165 ± 11*	16.1 ± 0.9*	1.6 ± 0.2	5
One-way ANOVA	P = 0.654	**P < 0.001**	P = 0.661	

The degree of potentiation of glutamate-induced responses, recorded in the presence of a given compound at concentrations of 0.3-30 μM (for compounds **9-18**), 1-100 μM (for compounds **1-8** and **21-24**), and 1-300 μM (for compounds **19** and **20**) was determined in individual cells for at least five compound doses and the data were fitted to the Equation (2). Statistical tests on the potency of compounds (**2-8**, **9-13**, **14-18** and **19-24**) were performed on $\log EC_{50}$ and logHill values (One-way ANOVA); $P \leq 0.05$ was used for the determination of significance, as marked in bold. Unpaired Student's t-test was used for statistical comparisons of E_{max} and $\log EC_{50}$ values for compounds **2-24** to **PES** (**1**;*P = 0.001− 0.050; **P < 0.001).

The series of 20-oxo-pregn-5-enes (**2-8**) exhibits similar values of E_{max} within this structural family (from 93% for the least efficacious compound **2** to 191% for the most efficacious

compound **7**). In contrast, the EC_{50} values for compounds **2-8** differ significantly, with the EC_{50} of the most potent compound **6** (**Figure 4A**) being up to 6-fold lower (8.5 μM) than that of the least potent compound **2** (48.5 μM). The dose-response analysis of compounds **4-7** indicated that at a concentration of 100 μM these compounds potentiated more than predicted from the fit of their potentiating effect at concentrations of 1-30 μM. The potentiating effect induced by these compounds at a concentration of 100 μM was: 267 ± 47% (n = 4) for compound **4**, 460 ± 17% (n = 4) for compound **5**, 614 ± 56% (n = 10) for compound **6**, and 458 ± 119% (n = 4) for compound **7**. The reason for this increased potentiation at high compound concentrations is unknown and we did not analyze it in detail. Augmented potentiation was not observed for compounds **1-3** and **8-24** (100 μM).

To evaluate the role of the 20-oxo group at the steroid D-ring for biological activity at NMDARs, a series of pregn-5-enes (**9-13**) was synthesized. Interestingly, within this structural family, compounds exhibited more than 4-fold difference in the E_{max} values (48% for the least efficacious compound **13** compared to 209% for the most efficacious compound **11**; **Figure 4B**), with EC_{50} values varying from 9.9 μM for the most potent compound **11** to 26.2 μM for the least potent compound **13**.

Further, we analyzed a series of androst-5-enes (**14-18**) lacking a substituent at the position C-17 of the steroidal D-ring. Compound **15** (AND-hSuc) was established as the most efficacious PAM within this structural family with E_{max} value of 452% (**Figure 4C**), approximately 4.5-times higher than that of the least efficacious compound **18** (109%). In general, compounds **14-18** were recognized as the most potent PAMs of all the tested compounds **2-24**, with EC_{50} values varying from 1.8 μM for the most potent compound **18** to 7.4 μM for the least potent compound **15** from this series (**Table 2**). This structural modification led to a significant

improvement in potency for all compounds **14-18**, suggesting that non-substituted D-ring drives the activity of PAM toward stronger potency.

Finally, we tested a series of 17-oxo analogues (**19-24**) with 17-oxo group at the steroidal D-ring. 17-Oxo analogues exhibited similar values of E_{max} within this structural family (varying from 154% for the least efficacious compound **19** to 226% for the most efficacious compound **23**; **Figure 4D**). The potency of compounds **19-24** differs significantly, exhibiting up to 9-fold decrease in EC_{50} value for the most potent compound **24** (16.1 μM) compared to the least potent compound **19** (151.4 μM).

Computational Estimate of Physicochemical Properties of Compounds 2-24.

Our recent results for steroids with inhibitory action at NMDARs showed the plasma membrane as the route for the steroid to reach its binding site on the receptor.[23] We could speculate that steroids with a potentiating effect, which are structurally similar to the inhibitory ones, may employ the same route. Therefore, we have analyzed the role of lipophilicity in the potency of compounds at NMDARs.

Computational analysis was used to evaluate the lipophilic qualities of compounds **1-24**. The relevant physicochemical properties (ΔG_{solv}, logP and logD values), which are commonly used for describing the compound lipophilicity,[24] were calculated by quantum mechanics computational methods and with a physicochemical properties predictor.[25] The computational results are summarized in **Table S1** (for details see Supporting Information). Compounds **5** and **14** show similar values of logD (1.29 and 1.28, respectively). However, the EC_{50} value for compound **5** (19.0 μM) is 4-times higher than for compound **14** (4.7 μM). Similarly, more lipophilic compounds **11** and **22** (logD values of 2.47 and 2.42, respectively) show an

approximately five-fold difference in EC_{50} values (**Table 2**). These results suggest that compound lipophilicity and presumed solubility in the membrane play only a minor role in determining the compound potency and indirectly indicate that potency is more dependent on the specific (structural/binding) interactions between the compound and the NMDAR.

Effect of 20-Oxo-PE-hAdi (6) on Recombinant GluN1/GluN2A-D and Native Receptors.

Compound **6** was selected from the library of compounds **2**-**24** for further biological evaluation. To investigate the subunit selectivity of compound **6**, cDNAs coding for the GluN1 and GluN2A-D subunits were co-transfected into HEK293 cells. Dose-response analysis for the effect of compound **6** on GluN1/GluN2A-D receptors showed no significant difference in the efficacy and potency at receptors differing in the subunit composition (**Table 3**).

Subsequently we compared the effect of compound **6** (30 μM) and **PES** (100 μM) at recombinant GluN1/GluN2A-D receptors. The results (**Figure 5**) indicate that compound **6** (30 μM) potentiated all tested subunit compositions of GluN1/GluN2A-D receptors (the degree of potentiation was: 192 ± 21% for GluN1/GluN2A, 137 ± 11% for GluN1/GluN2B, 118 ± 17% for GluN1/GluN2C, and 88 ± 11% for GluN1/GluN2D; $n = 6$). **PES** (100 μM) potentiated GluN1/GluN2A-B receptor responses (107 ± 17% ($n = 4$) for GluN1/GluN2A and 78 ± 4% ($n = 13$) for GluN1/GluN2B) while it inhibited GluN1/GluN2C-D receptor responses (-47 ± 4% for GluN1/GluN2C and -48 ± 3% for GluN1/GluN2D; $n = 4$). This agrees well with the results of previous experiments showing that **PES** has a dual potentiating and inhibitory action at NMDARs.[22,26] The predominant effect of **PES** (potentiation of GluN1/GluN2A-B and inhibition of GluN1/GluN2C-D) is the result of different efficacy rather than potency, which is higher for

GluN1/GluN2C-D receptors than for GluN1/GluN2A-B receptors.[26] In our experiments we have seen no evidence of a dual effect for compound **6**.

Table 3. Summary of the dose-response analysis data for the potentiation effect of compound **6** on responses of GluN1/GluN2A-D receptors.

Receptor	**E_{max} ± SEM (%)**	**EC_{50} ± SEM (μM)**	***h* ± SEM**	***n***
GluN1/GluN2A	203 ± 43	8.9 ± 1.4	1.7 ± 0.2	7
GluN1/GluN2B	157 ± 16	9.1 ± 1.1	1.7 ± 0.1	8
GluN1/GluN2C	145 ± 39	10.4 ± 3.5	1.7 ± 0.2	6
GluN1/GluN2D	115 ± 9	13.0 ± 1.9	1.7 ± 0.3	5
One-way ANOVA	P = 0.363	P = 0.520	P = 0.997	

The degree of potentiation of glutamate-induced responses recorded in the presence of compound **6** (1-30 μM) was determined in individual cells, and data were fit to the Equation (2). Statistical tests on the compound **6** potency were performed on logEC_{50} and logHill values (One-way ANOVA).

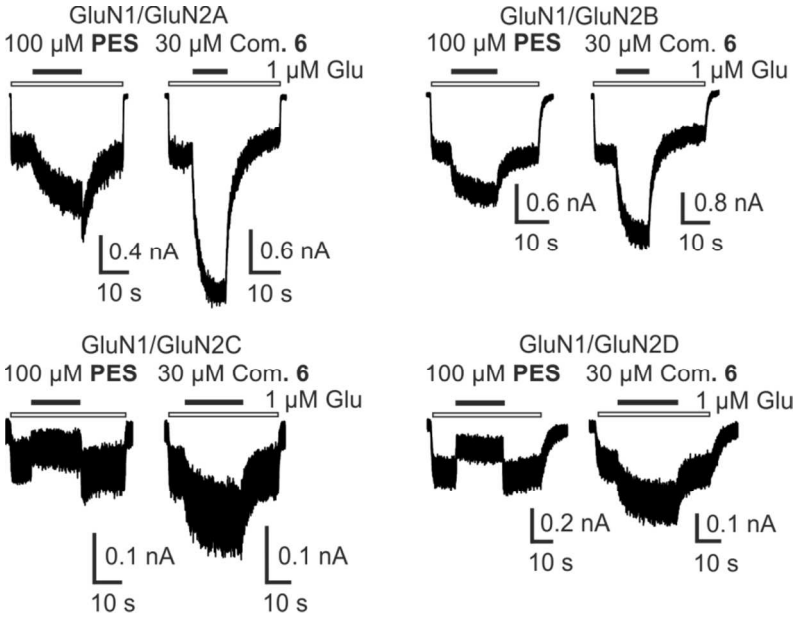


Figure 5. Comparison of the effect of **PES** and compound **6** on GluN1/GluN2A-D receptor responses. Examples of traces obtained from HEK293 cells transfected with cDNAs encoding for GluN1 and GluN2A-D subunits. **PES** (100 μM) and compound **6** (30 μM) were applied simultaneously with 1 μM glutamate (the duration of the compound and glutamate application is indicated by filled and open bars, respectively).

Native NMDARs expressed in cultured hippocampal neurons were potentiated (138 ± 11%, n = 6; **Figure 6**) by compound **6** (30 μM) to a similar extent as recombinant GluN1/GluN2B receptors (136%; **Figure 2A**; P = 0.824). In contrast to the relatively pronounced potentiating effect of compound **6** on native and recombinant NMDARs responses, compound **6** (30 μM) had only a small inhibitory effect (-7 ± 2%, n = 6) on responses induced by selective AMPARs agonist (AMPA 5 μM) recorded in the presence of cyclothiazide (10 μM) for reducing receptor desensitization.[27] Compound **6** (30 μM) inhibited responses induced by 5 μM GABA (-90 ± 2%, n = 5). These effects of compound **6** are qualitatively similar to **PES** effects at native NMDARs, AMPARs, and $GABA_A$Rs.[13,28]

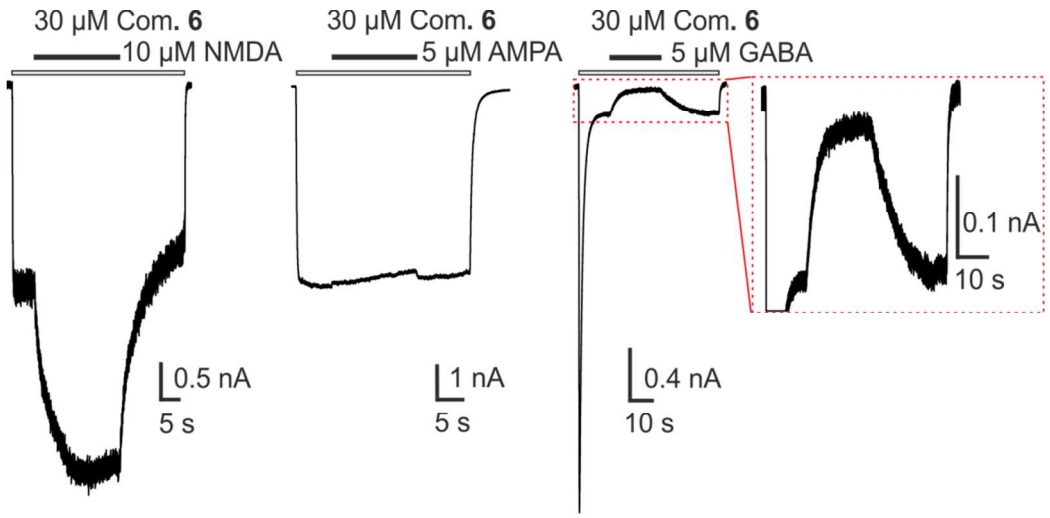


Figure 6. The effect of compound **6** on native NMDARs, AMPARs, and $GABA_A$Rs. Examples of traces obtained from cultured hippocampal neurons. Compound **6** (30 μM) was applied simultaneously with NMDA (10 μM), AMPA (5 μM) and GABA (5 μM) (the duration of compound and agonist application is indicated by filled and open bars, respectively).

Conclusions

In this study, we examined a library of compounds **2-24**, bearing a C-3 hemiester moiety, Δ^5-double bond and various modifications at position C-17, in order to evaluate their ability to modulate the activity of NMDARs. The results of our experiments indicate that the C-17 substituent of the D-ring can be structurally modified or fully degraded while maintaining a positive modulatory effect of the steroid. We have also shown that the potentiating effect of these compounds exhibits a dependency on the length of the C-3 substituent for each D-ring modification. The most efficacious and potent modulators, respectively, from all tested compounds (**2-24**), were compound **15**, exhibiting the E_{max} value of 452%, and compound **18**, exhibiting the EC_{50} value of 1.8 μM. In addition, we have shown that the selected compound **6**

has a subunit-independent effect at recombinant NMDARs, which is similar to that at native NMDARs, and has only a minor inhibitory effect at AMPARs. Our data further indicate that compound **6** is an inhibitor of native $GABA_A$Rs. In summary, we conclude that **PES** analogues modified at the C-3 and/or at the D-ring offer new prospects for further optimization of pharmacological and pharmacokinetic properties of these neuroactive compounds.

Experimetal Section

Chemistry.

General. Melting points were determined with a Hund/Wetzlar micromelting point apparatus (Germany) and are uncorrected. Optical rotations were measured in chloroform using an Autopol IV (Rudolf Research Analytical, Flanders, USA). $[\alpha]_D$ values are given in deg (10^{-1} deg cm^2 g^{-1}). IR spectra were recorded in a Bruker IFS 55 spectrometer (wavenumbers in cm^{-1}). Proton and carbon NMR spectra were measured in a Bruker AVANCE-400 FT NMR spectrometer (400 MHz, 101 MHz) in $CDCl_3$, with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ scale). Coupling constants (J) and widths of multiplets (W) are given in Hz. High-resolution MS spectra were performed with a Q-Tof microspectrometer (Waters). Elemental analysis was performed on a Perkin-Elmer 2400 Series II CHNS/O Analyzer (USA). Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals). For column chromatography, neutral silica gel 60 μm (Merck) was used. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. The purity of the final compounds was assessed by a combination of NMR and on the basis of LC-HR-MS analysis or elemental analysis, and the results showed they were greater than 95%.

Biological Activity.

Electrophysiological experiments were performed on HEK293 cells transfected with cDNA containing GluN1-1a/GluN2A-D/GFP genes and on cultured hippocampal neurons as described previously.[23a,29] Glutamate-induced responses were recorded at a holding potential of -60 mV. Whole-cell voltage-clamp recordings were made with a patch-clamp amplifier (Axopatch 200B; Axon Instruments. Inc., Foster City, CA) after series resistance (<10 MΩ) and capacitance

compensation of 80-90%. For the application of test and control solutions, a microprocessor-controlled multibarrel fast-perfusion system was used, with a time constant of solution exchange around the cells of ~10 ms. Agonist-induced responses were low-pass filtered at 2 kHz, digitally sampled at 5 kHz, and analyzed with pClamp software version 9.2 (Molecular Devices). Patch pipettes (3–5 MΩ) pulled from borosilicate glass were filled with a Cs^+-based intracellular solution- containing the following (in mM): 120 gluconic acid δ-lactone, 15 CsCl, 10 BAPTA, 10 HEPES, 3 $MgCl_2$, 1 $CaCl_2$ and 2 ATP-Mg salt (pH-adjusted to 7.2 with CsOH). Extracellular solution (ECS) contained the following (in mM): 160 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, 0.7 $CaCl_2$ and 0.2 EDTA (pH-adjusted to 7.3 with NaOH). NMDAR responses were induced by 1 μM or 1 mM glutamate (in recombinant receptors) and 10 μM NMDA (native receptors) together with 10 μM glycine. The ECS used for native receptors had the same composition as the ECS used for recombinant receptors. For native NMDAR recordings, ECS additionally contained 10 μM CNQX, 10 μM bicuculline and 0.5 μM TTX. AMPAR currents were induced with 5 μM AMPA and the ECS additionally contained 50 μM D-AP5, 10 μM bicuculline, 0.5 μM TTX and 10 μM cyclothiazide. $GABA_AR$ responses were induced with 5 μM GABA and the ECS additionally contained 50 μM D-AP5, 10 μM CNQX and 0.5 μM TTX. Compound solutions were prepared fresh before each experiment as a stock solution in dimethyl sulfoxide (DMSO). The same concentration of DMSO (1%) was maintained in all test and control extracellular solutions. Experiments were performed at room temperature (21–25 °C).

Experimental Data for Compounds 1-28

General procedure I: Synthesis of steroidal hemiesters from anhydrides

A mixture of steroid **25**, **26**, **27**, or **28** (1 mmol) and dicarboxylic acid anhydride (6 equiv) was dried overnight at 50 °C. Dry pyridine (12 mL) and DMAP (2.4 equiv) were added. The mixture was heated at 110 °C for 6 h under an inert atmosphere. It was then poured into water and extracted with DCM. Combined organic extracts were washed with brine and dried. Solvents were evaporated and the residue was purified on a column of silica gel.

General procedure II: Synthesis of steroidal hemiesters from ω-dicarboxylic acids

A solution of steroid **25**, **26**, **27**, or **28** (1 mmol) in dry DCM (5 mL) was added under an inert atmosphere to a solution of dicarboxylic acid (2 mmol), EDCI (2 mmol), DIPEA (2 mmol), and DMAP (2.4 equiv) in dry DCM (10 mL) at 0 °C under stirring. The reaction mixture was stirred at room temperature for 18 h. The solvents were then evaporated and the residue was dissolved with DCM, washed with water, dried over sodium sulfate, and concentrated *in vacuo*. Purification on a column of silica gel gave the desired steroidal hemiester.

20-Oxo-pregn-5-en-3β-yl sulfate Pyridinium Salt (1, PES). Sulfur trioxide pyridine complex (318 mg, 2 mmol) was added to a solution of compound **25** (316 mg, 1 mmol) in chloroform (5 mL, P_2O_5 dried and distilled) and dry pyridine (1 drop). The reaction mixture was stirred at room temperature under an inert atmosphere for 6 h. The progress of the reaction was checked by TLC. The reaction mixture was then allowed to stand at −18 °C for 2 h and the solids were filtered off through a plug of cotton wool. The filtrate was evaporated and dried (25 °C, 100 Pa)

for 1 h. The crude material was dissolved in freshly dried chloroform (4 mL), allowed to stand at −18 °C for 2 h, and the solid was filtered off. The filtrate was evaporated, yielding compound **1** (425 mg, 89%): m.p. 182–184 °C ($CHCl_3$); $[\alpha]_D^{20}$ 18.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CHCl_3$): δ 0.64 (3H, s, H-18), 1.02 (3H, s, H-19), 2.14 (3H, s, H-21), 2.55 (1H, t, J = 8.9, H-17), 2.65 (1H, ddd, J_1 = 2.3, J_2 = 5.1, J_3 = 13.3, H-4), 4.37 (1H, tt, J_1 = 4.8, J_2 = 11.6, H-3), 5.40 (1H, d, J = 5.2, H-6), 8.02 (2H, m, H-2′ and H-4′, pyridinium), 8.49 (1H, m, H-3′, pyridinium), 9.00 (2H, m, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.7 (C=O), 145.7 (C-1′ and C-5′, pyridinium), 142.5 (C-3′, pyridinium), 140.3 (C-5), 127.2 (C-2′ and C-4′, pyridinium), 122.2 (C-6), 78.9 (C-3), 63.8, 57.0, 50.0, 43.9, 39.3, 38.9, 37.3, 36.6, 31.9, 31.9, 31.7, 28.9, 24.6, 22.9, 21.2, 19.4, 13.3. IR ($CHCl_3$): 1698 (C=O); 1665 (C=C); 1490 (pyridinium); 971, 952 (COS). MS: ESI *m/z* 395.3 (100%, M – pyH). HR-MS (ESI) *m/z*: for $C_{21}H_{31}O_5S$ [M – pyH] calcd, 395.18977; found, 395.19906.

20-Oxo-pregn-5-en-3β-yl hemioxalate (2, 20-Oxo-PE-hOxa).[9a] A mixture of compound **25** (316 mg, 1 mmol), dry DCM (5 mL), triethylamine (0.15 mL), and two drops of previously prepared solution (2 mL of dry DCMDCM and 1 drop DMF) was added to a cooled (0 °C) mixture of DCM (5 mL) and oxalyl chloride (0.26 mL, 3 mmol). The mixture was allowed to reach 10 °C and then stirred for 2 h under these conditions. 20 mL of water was then added to decompose the excess reagent, and the mixture was stirred for 30 min at room temperature. The organic layer was separated and ethyl acetate (20 mL) was added to the DCM extract. An aqueous solution of potassium carbonate was then added (10%, 50 mL), the organic layer with undesired by-products was separated, and then an aqueous solution of HCl was cautiously added to the aqueous layer (1N, to pH ~4). The crude product was obtained by extraction with ethyl

acetate (2 x 25 mL) and dried. Chromatography on a silica gel column (5% methanol in DCM) gave **2** (140 mg, 36%): m.p. 189–191 °C (ethyl acetate/*n*-heptane); $[\alpha]_D^{20}$ 6.2 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18), 1.04 (3H, s, H-19), 2.14 (3H, s, H-21), 2.54 (1H, t, *J* = 8.9, H-17), 4.81 (1H, m, H-3), 5.43 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 210.4 (C=O), 157.9 (COOH), 157.7 (COO), 138.8 (C-5), 123.4 (C-6), 78.3 (C-3), 63.8 (C-17), 56.9, 49.9, 44.2, 38.8, 37.6, 36.9, 36.7, 31.9, 31.8, 31.7, 27.4, 24.6, 22.9, 21.1, 19.3, 13.3. IR ($CHCl_3$): 1801, 1760, 1699 (C=O); 1672 (C=C); 1192 (C-O). MS: ESI *m/z* 387.2 (100%, M – 1), 388.2 (24%, M). HR-MS (ESI) *m/z*: for $C_{23}H_{31}O_5$ [M – 1] calcd, 387.21660; found, 387.21676.

20-Oxo-pregn-5-en-3β-yl hemimalonate (3, 20-Oxo-PE-hMal). Dry toluene (15 mL) was added to a mixture of compound **25** (316 mg, 1 mmol) and 2,2-dimethyl-4,6-dioxo-1,3-dioxolane (Meldrum´s acid, 171 mg, 1.5 mmol). The mixture was heated at 80 °C for 48 h under an inert atmosphere. It was then washed with brine and the solvent was evaporated. Chromatography of the residue on a silica gel column (30-50% ethyl acetate in petroleum ether) gave **3** (149 mg, 37%): m.p. 166–168 °C (acetone); $[\alpha]_D^{20}$ 10.6 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18), 1.02 (3H, s, H-19), 2.13 (3H, s, H-21), 2.54 (1H, t, *J* = 8.8, H-17), 3.42 (2H, s, H-2´), 4.71 (1H, m, H-3), 5.39 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.9 (C=O), 169.5 (COOH), 167.4 (COO), 139.3 (C-5), 122.9 (C-5), 76.0 (C-3), 63.8 (C-17), 56.9, 49.9, 44.1, 40.4, 38.9, 37.9, 37.0, 36.7, 31.9, 31.9, 31.7, 27.6, 24.6, 22.9, 21.1, 19.4, 13.3. IR ($CHCl_3$): 1746, 1736, 1725, 1706 (C=O); 1675 (C=C); 1192 (C-O). MS: ESI *m/z* 357.3 (100%, M – 1 – CO_2), 401.3 (10%, M – 1). HR-MS (ESI) *m/z*: for $C_{24}H_{33}O_5$ [M – 1] calcd, 401.23335; found, 401.23291.

20-Oxo-pregn-5-en-3β-yl hemisuccinate (4, 20-Oxo-PE-hSuc).[9a] Compound **4** was prepared according to General procedure I. Starting from compound **25** (251 mg, 0.79 mmol), compound **4** (172 mg, 52%) was obtained as a white solid by column chromatography (30-50% ethyl acetate in petroleum ether): m.p. 161–163 °C (toluene); $[\alpha]_D^{20}$ +14.3 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (s, 3H, H-18), 1.02 (3H, s, H-19), 2.12 (3H, s, H-21), 2.61–2.68 (4H, m, $OOCCH_2CH_2COO$), 4.62 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.8 (C=O), 177.0 (COOH), 171.7 (COO), 139.6 (C-5), 122.5 (C-6), 74.5 (C-3), 63.8, 56.9, 50.0, 44.1, 38.9, 38.1, 37.1, 36.7, 31.9, 31.9, 31.7, 29.3, 28.9, 27.8, 24.6, 22.9, 21.1, 19.4, 13.3. IR ($CHCl_3$): 1731, 1716 (C=O); 1671 (C=C). MS: ESI *m/z* 415.3 (100%, M – 1). For $C_{25}H_{36}O_5$ (416.5) calcd: 72.08%, C; 8.71%, H. Found: 71.85%, C; 8.63%, H.

20-Oxo-pregn-5-en-3β-yl hemiglutarate (5, 20-Oxo-PE-hGlu).[9a] Compound **5** was prepared according to General procedure I. Starting from compound **25** (316 mg, 1 mmol), compound **5** (219 mg, 51%) was obtained as a white solid by column chromatography (2-5% acetone in DCM): m.p. 137–139 °C (methanol); $[\alpha]_D^{20}$ 9.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18), 1.02 (3H, s, H-19), 1.96 (2H, p, *J* = 7.3, H-3′), 2.12 (3H, s, H-21), 2.37 (2H, t, *J* = 7.3, H-2′), 2.43 (2H, t, *J* = 7.3, H-4′), 2.54 (1H, t, *J* = 8.9, H-17), 4.63 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.8 (C=O), 177.9 (COOH), 172.4 (COO), 139.7 (C-5), 122.5 (C-6), 74.1 (C-3), 63.8 (C-17), 56.9, 50.0, 44.1, 38.9, 38.2, 37.1, 36.7, 33.6, 32.9, 31.9, 31.9, 31.7, 27.8, 24.6, 22.9, 21.1, 20.0, 19.4, 13.3. IR ($CHCl_3$): 1725, 1711 (C=O); 1655 (C=C); 1417 (C-O). MS: ESI *m/z* 429.3 (100%, M – 1), 430.3 (30%, M). For $C_{26}H_{38}O_5$ (430.3) calcd: 72.53%, C; 8.90% H. Found: 72.36%, C; 8.89%, H.

20-Oxo-pregn-5-en-3β-yl hemiadipate (6, 20-Oxo-PE-hAdi). Compound **6** was prepared according to General procedure II. Starting from compound **25** (316 mg, 1 mmol), compound **6** (187 mg, 42%) was obtained as a white solid by column chromatography (2-5% acetone in DCM): m.p. 135–136 °C (ethyl acetate/*n*-heptane); $[\alpha]_D^{20}$ 3.0 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18), 1.02 (3H, s, H-19), 2.12 (3H, s, H-21), 2.28–2.40 (4H, m, H-adipate), 2.53 (1H, t, *J* = 9.0, H-17), 4.63 (1H, m, H-3), 5.38 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.7 (C=O), 178.0 (COOH), 172.8 (COO), 139.8 (C-5), 122.5 (C-6), 73.9 (C-3), 63.8 (C-17), 56.9, 50.0, 44.1, 38.9, 38.2, 37.1, 36.7, 34.3, 33.5, 31.9, 31.9, 31.7, 27.9, 24.6, 24.5, 24.2, 22.9, 21.2, 19.4, 13.3. IR ($CHCl_3$): 1730, 1709 (C=O); 1655 (C=C); 1417 (C-O). MS: ESI *m/z* 443.3 (100%, M – 1), 444.3 (25%, M). For $C_{27}H_{40}O_5$ (444.3) calcd: 72.94%, C; 9.07% H. Found: 72.71%, C; 9.01%, H.

20-Oxo-pregn-5-en-3β-yl hemipimelate (7, 20-Oxo-PE-hPim). Compound **7** was prepared according to General procedure II. Starting from compound **25** (316 mg, 1 mmol), compound **7** (215 mg, 47%) was obtained as a white solid by column chromatography (1-10% acetone in DCM): m.p. 123–125 °C (methanol); $[\alpha]_D^{20}$ 10.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18), 1.02 (3H, s, H-19), 2.12 (3H, s, H-21), 2.26–2.39 (4H, m, H-pimelate), 2.53 (1H, t, *J* = 8.9, H-17), 4.63 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.7 (C=O), 178.5 (COOH), 173.1 (COO), 139.8 (C-5), 122.4 (C-6), 73.8 (C-3), 63.8 (C-17), 56.9, 50.0, 44.1, 38.9, 38.2, 37.1, 36.7, 34.5, 33.7, 31.9, 31.9, 31.7, 28.6, 27.9, 24.7, 24.6, 24.4, 22.9, 21.1, 19.4, 13.3. IR ($CHCl_3$): 1727, 1708 (C=O); 1655 (C=C); 1416 (C-O). MS: ESI *m/z* 457.3 (100%, M – 1), 458.3 (32%, M). For $C_{28}H_{42}O_5$ (458.3) calcd: 73.33%, C; 9.23% H. Found: 73.05%, C; 9.44%, H.

20-Oxo-pregn-5-en-3β-yl hemisuberate (8, 20-Oxo-PE-hSub). Compound **8** was prepared according to General procedure II. Starting from compound **25** (316 mg, 1 mmol), compound **8** (208 mg, 44%) was obtained as a white solid by column chromatography (1-10% acetone in DCM): m.p. 105–107 °C (ethyl acetate/*n*-heptane); $[\alpha]_D^{20}$ 10.4 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18), 1.02 (3H, s, H-19), 2.12 (3H, s, H-21), 2.24–2.37 (4H, m, H-suberate), 2.53 (1H, t, *J* = 9.0, H-17), 4.63 (1H, m, H-3), 5.8 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.7 (C=O), 178.6 (COOH), 173.3 (COO), 139.8 (C-5), 122.4 (C-6), 73.7 (C-3), 63.8 (C-17), 56.9, 50.0, 44.1, 38.9, 38.2, 37.1, 36.7, 34.7, 33.9, 31.9, 31.9, 31.7, 28.8, 28.8, 27.9, 24.9, 24.6, 24.6, 22.9, 21.1, 19.4, 13.3. IR ($CHCl_3$): 1728, 1705 (C=O); 1655 (C=C); 1417 (C-O). MS: ESI *m/z* 471.3 (100%, M – 1), 472.3 (30%, M). For $C_{29}H_{44}O_5$ (472.3) calcd: 73.69%, C; 9.38% H. Found: 73.41%, C; 9.54%, H.

Pregn-5-en-3β-yl hemimalonate (9, PE-hMal). Compound **9** was prepared in the same manner as compound **3**. Starting from compound **26** (302 mg, 1 mmol), compound **9** (125 mg, 32%) was obtained as a white solid by column chromatography (50% ether in petroleum ether): m.p. 176–178 °C (methanol); $[\alpha]_D^{20}$ -41.7 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58 (3H, s, H-18), 0.87 (3H, t, *J* = 7.3, H-21), 1.03 (3H, s, H-19), 3.41 (2H, s, H-2′), 4.67–4.76 (1H, m, H-3), 5.40 (1H, d, *J* = 4.8, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.5 (COOH), 167.4 (COO), 139.2 (C-5), 123.3 (C-6), 76.3 (C-3), 56.2, 53.0, 50.5, 42.0, 40.4, 37.9, 37.9, 37.0, 36.8, 32.1, 31.9, 28.2, 27.7, 24.7, 23.1, 20.9, 19.4, 13.5, 12.5. IR ($CHCl_3$): 1757, 1737, 1718 (C=O); 1670 (C=C); 1409, 1288 (C-O). MS: ESI *m/z* 387.3 (27%, M – 1), 343.3 (100%, M – 1 – CO_2). HR-MS (ESI) *m/z*: for $C_{24}H_{35}O_4$ [M – 1] calcd, 387.25408; found, 387.25348.

Pregn-5-en-3β-yl hemisuccinate (10, PE-hSuc). Compound **10** was prepared according to General procedure I. Starting from compound **26** (302 mg, 1 mmol), compound **10** (145 mg, 43%) was obtained as a white solid by column chromatography (10-30% ethyl acetate in petroleum ether): m.p. 151–153 °C (methanol); $[\alpha]_D^{20}$ -36.8 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58 (s, 3H, H-18), 0.87 (3H, t, *J* = 7.3, H-21), 1.02 (3H, s, H-19), 2.58–2.70 (4H, m, $OOCCH_2CH_2COO$), 4.63 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.5 (COOH), 171.7 (COO), 139.7 (C-5), 122.9 (C-6), 74.7 (C-3), 56.3, 53.0, 50.6, 42.1, 38.2, 37.9, 37.1, 36.8, 32.1, 32.0, 29.3, 29.0, 28.2, 27.8, 24.7, 23.1, 20.9, 19.4, 13.5, 12.5. IR ($CHCl_3$): 1730, 1716 (C=O); 1670 (C=C); 1290 (C-O). MS: ESI *m/z* 401.3 (100%, M – 1), 402.3 (30%, M). HR-MS (ESI) *m/z*: for $C_{25}H_{37}O_4$ [M – 1] calcd, 401.26973; found, 401.26941.

Pregn-5-en-3β-yl hemiglutarate (11, PE-hGlu). Compound **11** was prepared according to General procedure I. Starting from compound **26** (302 mg, 1 mmol), compound **11** (184 mg, 44%) was obtained as a white solid by column chromatography (1% methanol in DCM): m.p. 128–130 °C (methanol); $[\alpha]_D^{20}$ -38.7 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58 (3H, s, H-18), 0.87 (3H, t, *J* = 7.3, H-21), 1.02 (3H, s, H-19), 1.96 (2H, p, *J* = 7.3, H-3′), 2.37 (2H, t, *J* = 7.3, H-2′), 2.43 (2H, t, *J* = 7.3, H-4′), 4.63 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0 (COOH), 172.4 (COO), 139.7 (C-5), 122.8 (C-6), 74.2 (C-3), 56.3, 53.0, 50.6, 42.0, 38.2, 37.9, 37.1, 36.8, 33.6, 32.9, 32.1, 32.0, 28.2, 27.9, 24.7, 23.1, 20.9, 20.0, 19.5, 13.5, 12.5. IR ($CHCl_3$): 1724, 1713 (C=O); 1671 (C=C); 1192 (C-O). MS: ESI *m/z* 439.3 (100%, M + Na), 440.3 (30%, M + Na + 1). HR-MS (ESI) *m/z*: for $C_{26}H_{40}O_4Na$ [M + Na] calcd, 439.28189; found, 439.28188.

Pregn-5-en-3β-yl hemiadipate (12, PE-hAdi). Compound **12** was prepared according to General procedure II. Starting from compound **26** (302 mg, 1 mmol), compound **12** (90 mg, 21%) was obtained as a white solid by column chromatography (0.3-1% methanol in DCM): m.p. 128–130 °C (methanol); $[\alpha]_D^{20}$ -36.2 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58 (3H, s, H-18), 0.87 (3H, t, *J* = 7.3, H-21), 1.02 (3H, s, H-19), 2.28–2.34 (4H, m, H-adipate), 4.63 (1H, m, H-3), 5.38 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.4 (COOH), 172.8 (COO), 139.8 (C-5), 122.8 (C-6), 74.1 (C-3), 56.3, 53.0, 50.6, 42.0, 38.3, 37.9, 37.1, 36.8, 34.3, 33.5, 32.1, 32.0, 28.2, 27.9, 24.8, 24.5, 24.2, 23.2, 20.9, 19.5, 13.5, 12.5. IR ($CHCl_3$): 1727, 1712 (C=O); 1670 (C=C); 1191 (C-O). MS: ESI *m/z* 429.3 (100%, M – 1), 430.3 (30%, M). HR-MS (ESI) *m/z*: for $C_{27}H_{41}O_4$ [M – 1] calcd, 429.30103; found, 429.30063.

Pregn-5-en-3β-yl hemipimelate (13, PE-hPim). Compound **13** was prepared according to General procedure II. Starting from compound **26** (302 mg, 1 mmol), compound **13** (157 mg, 35%) was obtained as a white solid by column chromatography (10-20% ethyl acetate in petroleum ether): m.p. 97–99 °C (EtOAc); $[\alpha]_D^{20}$ -38.5 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, CDCl3): δ 0.57 (3H, s, H-18), 0.86 (3H, t, *J* = 7.3, H-21), 1.01 (3H, s, H-19), 2.23-2.36 (4H, m, H-pimelate), 4.60 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CHCl_3$) δ 177.0 (COOH), 173.2 (COO), 139.8 (C-5), 122.7 (C-6), 74.0 (C-3), 56.3, 53.1, 50.6, 42.0, 38.3, 37.9, 37.1, 36.8, 34.5, 33.7, 32.1, 32.0, 28.6, 28.2, 27.9, 24.8, 24.7, 24.5, 23.1, 20.9, 19.4, 13.4, 12.5. IR ($CHCl_3$): 1726, 1712 (C=O); 1671 (C=C); 1257, 1196, 1178 (C-O). MS: ESI *m/z* 443.4 (100%, M – 1), 444.4 (30%, M). For $C_{28}H_{44}O_4$ (444.6) calcd: 75.63%, C; 9.97% H. Found: 75.25%, C; 9.98%, H.

Androst-5-en-3β-yl hemimalonate (14, AND-hMal). Compound **14** was prepared in the same manner as compound **3**. Starting from compound **28** (274 mg, 1 mmol), compound **14** (46 mg, 13%) was obtained as a white solid by column chromatography (10-15% acetone in petroleum ether): m.p. 169–171 °C (ethyl acetate); $[\alpha]_D^{20}$ -53.5 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (3H, s, H-18), 1.03 (3H, s, H-19), 3.42 (2H, s, H-2′), 4.73 (1H, m, H-3), 5.40 (1H, d, *J* = 4.8, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.3 (COOH), 167.8 (COO), 139.2 (C-5), 123.3 (C-6), 76.4 (C-3), 54.9, 50.4, 40.7, 40.4, 40.2, 38.7, 37.9, 37.0, 36.8, 32.3, 32.2, 27.7, 25.7, 21.2, 20.6, 19.4, 17.3. IR ($CHCl_3$): 1774, 1736, 1718 (C=O); 1670 (C=C); 1197, 1179, 1160 (C-O). MS: ESI *m/z* 359.2 (17%, M – 1), 315.2 (100%, M – 1 – CO_2). HR-MS (ESI) *m/z*: for $C_{22}H_{31}O_4$ [M – 1] calcd, 359.22278; found, 359.22263.

Androst-5-en-3β-yl hemisuccinate (15, AND-hSuc). Compound **15** was prepared according to General procedure I. Starting from compound **28** (274 mg, 1 mmol), compound **15** (250 mg, 67%) was obtained as a white solid by column chromatography (1% methanol in DCM): m.p. 159–161 °C (methanol); $[\alpha]_D^{20}$ -63.6 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (s, 3H, H-18), 1.02 (3H, s, H-19), 2.56–2.71 (4H, m, $OOCCH_2CH_2COO$), 4.62 (1H, m, H-3), 5.38 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.3 (COOH), 171.7 (COO), 139.6 (C-5), 122.9 (C-6), 74.7 (C-3), 54.9, 50.4, 40.7, 40.4, 38.8, 38.2, 37.1, 36.8, 32.3, 32.2, 29.4, 29.0, 27.8, 25.7, 21.2, 20.6, 19.5, 17.4. IR ($CHCl_3$): 1755, 1730, 1717 (C=O); 1672 (C=C); 1176 (C-O). MS: ESI *m/z* 373.4 (100%, M – 1), 374.4 (28%, M). For $C_{23}H_{34}O_4$ (374.3) calcd: 73.76%, C; 9.15% H. Found: 73.93%, C; 9.25%, H.

Androst-5-en-3β-yl hemiglutarate (16, AND-hGlu). Compound **16** was prepared according to General procedure I. Starting from compound **28** (274 mg, 1 mmol), compound **16** (165 mg, 42%) was obtained as a white solid by column chromatography (0.2% methanol in DCM): m.p. 76–78 °C (methanol); $[\alpha]_D^{20}$ -52.0 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, H-18), 1.02 (3H, s, H-19), 1.95 (2H, p, *J* = 7.3, H-3′), 2.37 (2H, t, *J* = 7.3, H-2′), 2.43 (2H, t, *J* = 7.3, H-4′), 4.63 (1H, m, H-3), 5.38 (1H, d, *J* = 5.4, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.6 (COOH), 172.4 (COO), 139.7 (C-5), 122.8 (C-6), 74.2 (C-3), 54.9, 50.4, 40.7, 40.4, 38.8, 38.3, 37.2, 36.8, 33.6, 33.0, 32.3, 32.2, 27.9, 25.7, 21.2, 20.65, 20.06, 19.5, 17.4. IR ($CHCl_3$): 1725, 1713 (C=O); 1670 (C=C); 1417, 1285 (C-O). MS: ESI *m/z* 387.3 (100%, M – 1), 388.4 (30%, M). For $C_{24}H_{36}O_4$ (388.3) calcd: 74.19%, C; 9.34% H. Found: 74.23%, C; 9.32%, H.

Androst-5-en-3β-yl hemiadipate (17, AND-hAdi). Compound **17** was prepared according to General procedure II. Starting from compound **28** (274 mg, 1 mmol), compound **17** (140 mg, 35%) was obtained as a white solid by column chromatography (0.2-0.5% methanol in DCM): m.p. 86–88 °C (methanol); $[\alpha]_D^{20}$ -52.7 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, H-18), 1.02 (3H, s, H-19), 2.27–2.41 (4H, m, H-adipate), 4.63 (1H, m, H-3), 5.38 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.6 (COOH), 172.8 (COO), 139.8 (C-5), 122.8 (C-6), 74.1 (C-3), 54.9, 50.4, 40.7, 40.4, 38.8, 38.2, 37.2, 36.8, 34.3, 33.6, 32.3, 32.2, 27.9, 25.7, 24.5, 24.2, 21.2, 20.6, 19.5, 17.4. IR ($CHCl_3$): 1728, 1711 (C=O); 1670 (C=C); 1416, 1288 (C-O). MS: ESI *m/z* 401.3 (100%, M – 1), 402.3 (30%, M). For $C_{25}H_{38}O_4$ (402.3) calcd: 74.59%, C; 9.51% H. Found: 74.54%, C; 9.58%, H.

Androst-5-en-3β-yl hemipimelate (18, AND-hPim). Compound **18** was prepared according to General procedure II. Starting from compound **28** (274 mg, 1 mmol), compound **18** (174 mg, 42%) was obtained as a white solid by column chromatography (0.2-0.5% methanol in DCM): m.p. 61–63 °C (methanol); $[\alpha]_D^{20}$ -53.0 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, H-18), 1.02 (3H, s, H-19), 2.26–2.39 (4H, m, H-pimelate), 2.53 (1H, t, *J* = 8.9, H-17), 4.63 (1H, m, H-3), 5.37 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.8 (COOH), 173.1 (COO), 139.8 (C-5), 122.7 (C-6), 73.9 (C-3), 54.9, 50.4, 40.7, 40.4, 38.8, 38.3, 37.2, 36.8, 34.5, 33.7, 32.3, 32.2, 28.6, 27.9, 25.7, 24.7, 24.4, 21.2, 20.6, 19.5, 17.4. IR ($CHCl_3$): 1725, 1712 (C=O); 1670 (C=C); 1416, 1290 (C-O). MS: ESI *m/z* 415.3 (100%, M – 1), 416.3 (35%, M). For $C_{26}H_{40}O_4$ (416.3) calcd: 74.96%, C; 9.68% H. Found: 75.04%, C; 9.77%, H.

17-Oxo-androst-5-en-3β-yl hemiglutarate (19, 17-Oxo-AND-hGlu). Compound **19** was prepared according to General procedure I. Starting from compound **27** (288 mg, 1 mmol), compound **19** (234 mg, 58%) was obtained as a white solid by column chromatography (0.5% methanol in DCM): m.p. 126–128 °C (methanol); $[\alpha]_D^{20}$ 0.0 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, H-18), 1.05 (3H, s, H-19), 1.96 (2H, p, *J* = 7.5, H-3′), 2.38 (2H, t, *J* = 7.3, H-2′), 2.43 (2H, t, *J* = 7.3, H-4′), 4.63 (1H, m, H-3), 5.41 (1H, d, *J* = 5.4, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 221.7 (C-17), 177.7 (COOH), 172.3 (COO), 139.8 (C-5), 122.0 (C-6), 73.9 (C-3), 51.8, 50.2, 47.7, 38.2, 37.0, 36.8, 36.0, 33.6, 32.9, 31.6, 31.5, 30.9, 27.8, 22.0, 20.4, 20.0, 19.5, 13.7. IR ($CHCl_3$): 1731, 1714 (C=O); 1670 (C=C); 1415, 1289 (C-O). MS: ESI *m/z* 401.3 (100%, M – 1), 402.3 (30%, M). For $C_{24}H_{34}O_5$ (402.2) calcd: 71.61%, C; 8.51% H. Found: 71.47%, C; 8.50%, H.

17-Oxo-androst-5-en-3β-yl hemiadipate (20, 17-Oxo-AND-hAdi). Compound **20** was prepared according to General procedure II. Starting from compound **27** (288 mg, 1 mmol), compound **20** (130 mg, 31%) was obtained as a white solid by column chromatography (0.5% methanol in DCM): m.p. 154–156 °C (methanol); $[\alpha]_D^{20}$ 0.0 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, H-18), 1.05 (3H, s, H-19), 2.28–2.40 (4H, m, H-adipate), 4.63 (1H, m, H-3), 5.38 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 221.1 (C-17), 178.3 (COOH), 172.8 (COO), 140.0 (C-5), 122.0 (C-6), 73.8 (C-3), 51.8, 50.2, 47.7, 38.2, 37.0, 36.8, 36.0, 34.3, 33.6, 31.6, 31.5, 30.9, 27.8, 24.5, 24.2, 22.0, 20.4, 19.5, 13.7. IR ($CHCl_3$): 1731, 1713 (C=O); 1670 (C=C); 1415, 1289 (C-O). MS: ESI *m/z* 415.3 (100%, M – 1), 416.3 (30%, M). For $C_{25}H_{36}O_5$ (416.3) calcd: 72.08%, C; 8.71% H. Found: 72.09%, C; 8.87%, H.

17-Oxo-androst-5-en-3β-yl hemipimelate (21, 17-Oxo-AND-hPim). Compound **21** was prepared according to General procedure II. Starting from compound **27** (288 mg, 1 mmol), compound **21** (150 mg, 35%) was obtained as a white solid by column chromatography (0.3-1% methanol in DCM): m.p. 71–73 °C (methanol); $[\alpha]_D^{20}$ 1.0 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, H-18), 1.05 (3H, s, H-19), 2.26–2.39 (4H, m, H-pimelate), 4.63 (1H, m, H-3), 5.41 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 221.3 (C-17), 178.6 (COOH), 173.0 (COO), 140.0 (C-5), 122.0 (C-6), 73.7 (C-3), 51.8, 50.3, 47.7, 38.2, 37.0, 36.8, 36.0, 34.5, 33.7, 31.6, 31.5, 30.9, 28.6, 27.8, 24.7, 24.4, 22.0, 20.4, 19.5, 13.6. IR ($CHCl_3$): 1731, 1713 (C=O); 1670 (C=C); 1414, 1285 (C-O). MS: ESI *m/z* 429.4 (100%, M – 1), 430.4 (30%, M). For $C_{26}H_{38}O_5$ (430.3) calcd: 72.53%, C; 8.90% H. Found: 72.66%, C; 8.97%, H.

17-Oxo-androst-5-en-3β-yl hemisuberate (22, 17-Oxo-AND-hSub). Compound **22** was prepared according to General procedure II. Starting from compound **27** (288 mg, 1 mmol), compound **22** (220 mg, 50%) was obtained as a white solid by column chromatography (0.5% methanol in DCM): m.p. 123–125 °C (methanol); $[\alpha]_D^{20}$ 0.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-18), 1.05 (3H, s, H-19), 2.25–2.38 (4H, m, H-suberate), 4.61 (1H, m, H-3), 5.41 (1H, m, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 221.1 (C-17), 178.9 (COOH), 173.2 (COO), 140.0 (C-5), 121.9 (C-6), 73.6 (C-3), 51.8, 50.2, 47.6, 38.2, 37.0, 36.8, 36.0, 34.6, 33.8, 31.6, 31.5, 30.9, 28.8, 28.8, 27.8, 24.9, 24.6, 22.0, 20.4, 19.5, 13.6. IR ($CHCl_3$): 1731, 1712 (C=O); 1680 (C=C); 1248 (C-O). MS: ESI *m/z* 443.3 (100%, M – 1), 444.3 (35%, M). For $C_{27}H_{40}O_5$ (444.3) calcd: 72.94%, C; 9.07% H. Found: 72.77%, C; 9.18%, H.

17-Oxo-androst-5-en-3β-yl hemiazelate (23, 17-Oxo-AND-hAze). Compound **23** was prepared according to General procedure II. Starting from compound **27** (288 mg, 1 mmol), compound **23** (110 mg, 24%) was obtained as a white solid by column chromatography (10-15% acetone in petroleum ether): m.p. 86–87 °C (acetone); $[\alpha]_D^{20}$ 0.8 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, H-18), 1.05 (3H, s, H-19), 2.25–2.40 (4H, H-azelaic acid ester), 4.61 (1H, m, H-3), 5.40 (1H, d, *J* = 4.8, H-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 221.3 (C-17), 178.8 (COOH), 173.3 (COO), 140.1 (C-5), 121.9 (C-6), 73.6 (C-3), 51.8, 50.2, 47.6, 38.2, 37.0, 36.8, 36.0, 34.7, 33.9, 31.6, 31.5, 30.9, 29.0 (2x), 28.9, 27.8, 25.0, 24.7, 22.0, 20.4, 19.5, 13.7. IR ($CHCl_3$): 1731, 1711 (C=O); 1671 (C=C); 1191(C-O). MS: ESI *m/z* 457.3 (100%, M – 1), 458.3 (32%, M). For $C_{28}H_{42}O_5$ (458.3) calcd: 73.33%, C; 9.23% H. Found: 73.67%, C; 9.39%, H.

17-Oxo-androst-5-en-3β-yl hemisebacate (24, 17-Oxo-AND-hSeb). Compound **24** was prepared according to General procedure II. Starting from compound **27** (288 mg, 1 mmol), compound **24** (130 mg, 27%) was obtained as a white solid by column chromatography (15% acetone in petroleum ether): m.p. 96–98 °C (acetone); $[\alpha]_D^{20}$ 0.0 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, H-18), 1.05 (3H, s, H-19), 2.24–2.37 (4H, m, H-sebacic acid ester), 4.62 (1H, m, H-3), 5.40 (1H, m, H-6). ^{13}C NMR (101 MHz, $CHCl_3$): δ 221.3 (C-17), 178.7 (COOH), 173.4 (COO), 140.1 (C-5), 121.9 (C-6), 73.6 (C-3), 51.8, 50.3, 47.7, 38.2, 37.1, 36.9, 36.0, 34.8, 33.9, 31.6, 31.5, 30.9, 29.1, 29.1, 29.1, 27.8, 25.1, 24.7, 22.0, 20.4, 19.5, 13.7. IR ($CHCl_3$): 1731, 1711 (C=O); 1670 (C=C); 1189, 1178 (C-O). MS: ESI *m/z* 471.4 (100%, M – 1), 472.4 (35%, M). For $C_{29}H_{44}O_5$ (472.7) calcd: 73.69%, C; 9.38% H. Found: 73.53%, C; 9.43%, H.

3β-Hydroxy-pregn-5-en (26). Trimethylsilyl chloride (62 mL, 0.48 mol) was added dropwise to a stirred mixture of pregnenolone **25** (10 g, 0.03 mol) and zinc powder (52 g, 0.47 mol) in DCM and methanol (1:1, 300 mL) at 0 °C. The reaction mixture was stirred at room temperature. After 18 h, zinc was removed by filtration. The filtrate was poured into an aqueous saturated solution of sodium hydrogen carbonate; the product was extracted with chloroform, washed with 1M hydrochloric acid, water, and dried over anhydrous sodium sulfate. Evaporation of the solvents yielded 7.5 g (78%) of compound **26**, which was crystallized for further synthesis: m.p. 133−135 °C (ethyl acetate/*n*-heptane), lit.[30]: 133-134 °C; $[\alpha]_D^{20}$ -55.3 (*c* 0.3, $CHCl_3$),[31] -60 (*c* 2.95, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.58 (3H, s, H-18), 0.87 (3H, t, *J* = 7.3, H-21), 1.01 (3H, s, H-19), 3.53 (1H, m, H-3), 5.36 (1H, m, H-6). ^{13}C NMR (101 MHz, $CHCl_3$): δ 140.9 (C-5), 121.8 (C-6), 71.9 (C-3), 56.3, 53.1, 50.7, 42.4, 42.1, 38.0, 37.4, 36.7, 32.1, 32.0, 31.8,

28.2, 24.8, 23.1, 21.0, 19.5, 13.5, 12.5. IR spectrum ($CHCl_3$): 3609, 3462 (OH); 1668 (C=C); 1046 (C-O). MS: EI *m/z* 302.3 (50%, M), 284.3 (85%, M – H_2O). For $C_{21}H_{34}O$ (302.5) calcd: 83.38%, C; 11.33%, H. Found: 83.22%, C; 11.16%, H.

3β-Hydroxy-androst-5-en (28). Compound **28** was prepared in the same manner as compound **26.**[21] Starting from compound **27** (10 g, 0.03 mol), compound **28** (7.2 g, 75%) was obtained: m.p. 135−136 °C (ethyl acetate/*n*-heptane), lit.[21], (133−134 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (3H, s, H-18), 1.01 (3H, s, H-19), 3.53 (1H, m, H-3), 5.36 (1H, m, H-6). ^{13}C NMR (101 MHz, $CHCl_3$): δ 140.9 (C-5), 121.9 (C-6), 71.9 (C-3), 55.0, 50.5, 42.4, 40.7, 40.4, 38.8, 37.4, 36.8, 32.3, 32.3, 31.8, 25.7, 21.3, 20.6, 19.6, 17.4.

Corresponding Author

* Mailing address: Eva Kudova, Department of Steroidal Inhibitors, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 2, Prague 6 – Dejvice, 16610, Czech Republic. Phone: +420-220-183-273. Fax: +420-220-183-578. Email: kudova@uochb.cas.cz.

Author Contributions

Synthesis was done by B.S. and H.CH.; *in vitro* screening was done by B.K., P.H. and V.V.; the computational analysis was done by M.N; written by E.K., L.V. and B.K.

Supporting Information. Computational analysis for compounds **1-24** and molecular string file for target compounds (CVS). This material is available free of charge via the Internet at http://pubs.acs.org.

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