The Ugi-Smiles reaction on steroids: Discovery of bioactive *N,N*-disubstituted 3-aminoestrones.

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ABSTRACT

The Ugi-Smiles multicomponent reaction is a powerful tool for obtaining *N*-arylamines from acidic phenols and has been widely used for gaining access to structurally diverse scaffolds. In this work we demonstrate that this isocyanide-based coupling can be used for the straightforward and efficient synthesis of *N,N*-disubstituted 3-aminoestrones, steroidal derivatives that usually show interesting biological activities. In this sense, we analyzed the scope and limitations of the reaction when applied to aromatic nitrosteroids and found that the outcome is highly influenced by the steric effects imposed by the steroidal skeleton. After optimization of the reaction conditions a set of thirteen N-substituted 3-aminoestromes were obtained, some of them with interesting antiproliferative and antiviral activities.

INTRODUCTION

The discovery of lead structures is central to the drug development process, and despite the advances on the synthesis of large compound libraries and on high-throughput screening technologies, finding new biological active molecules that might help in fighting against deadly diseases remains a slow pursuit, mainly because the structural factors necessary to create compound collections with a potent and specific biochemical activity are not fully understood.[1]

In the last years the phenotypic screening of libraries on cell-based or model organism-based assays, an approach reminiscent of the pre-genomics pharmacology, has reemerged as an alternative platform for drug discovery, [2,3] but this approach usually relies on focused compound collections having improved biological relevance, where structural diversity among the members of the library is paramount. However, the design and synthesis of small-molecule libraries with maximized molecular diversity represent a key challenge. This is where multicomponent reactions (MCRs) play a central role: MCRs are powerful, highly convergent synthetic procedures that might help to overcome the synthetic costs associated to the efficient introduction of structural diversity, as most of these reactions are operationally simple and are especially useful for generating, in few steps, diverse and complex scaffolds.

In this sense, the Ugi four-component reaction (U-4CR) is probably the most powerful MCR and occurs when a carbonyl compound, an amine, a carboxylic acid and an isocyanide react together to give an α-aminoacylamide.[4]

The versatility of the U-4CR has prompted many efforts seeking to expand the nature of the components which take part in it. In this context, El Kaïm et al. reported an Ugi-type four-component reaction, known as the Ugi-Smiles reaction[5], where the carboxilic component is replaced by an acidic phenol (such as nitrophenol) to yield *N*-aryl carboxamides (Figure 1)

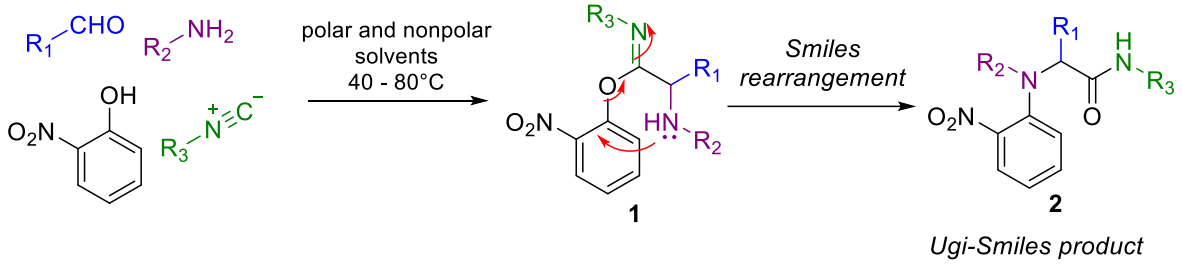


Figure 1. Ugi-Smiles reaction

In the Ugi-Smiles reaction the four components react to give an O-aryl imidate (**1**) which suffers a Smiles rearrangement that leads to the stable product **2**. This reaction works well for a wide range of substrates, specially the acidic component. In addition to *o*- and *p*-nitrophenols having different substituent patterns[6,7], alternative acidic components have been used: heteroatomic phenols[8], thiophenols[6,9], and others sulfur

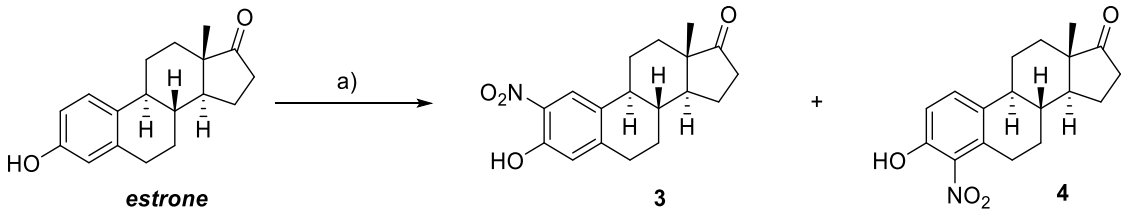
containing substrates (as S-bencylthiouracils[10] and saccharin[11]). Through the Ugi-Smiles reaction all these substrates afforded novel structures, some of them showing interesting biological activities such as antimalarial[12], psychoactive agents[13] and antiproliferative[14].

Aimed by our interest on the use of MCRs as key step in the structural diversification of steroids, we envisioned that the Ugi-Smiles reaction could be an appealing entry to 3-aminoestrones, which although being steroid derivatives with promising biological properties such as estrogenic, antiangiogenic and antitumoral[15,16], cannot be easily synthetized from readily available starting materials, as the scarce reports available imply photo-redox catalysis[17], electro-oxidative C-H/N-H couplings, Pd catalysis[15,16,18,19] or radical reactions.[20]

In this work we describe a preliminary study on the use of the Ugi-Smiles reaction to afford *N,N*-disubstituted 3-aminoestrones in which we have explored the most suitable reaction conditions, the scope of the procedure and discussed the particular effects that the steroidal scaffold sets on the outcome of the reaction. Moreover, a preliminary phenotypic screening of the resulting compounds showed that some of them exert promising biological activities *in vitro*.

RESULTS AND DISCUSSION

Firstly, 2-nitroestrone (**3**) and 4-nitroestrone (**4**) were obtained by direct nitration of commercially available estrone according to a reported procedure[21].

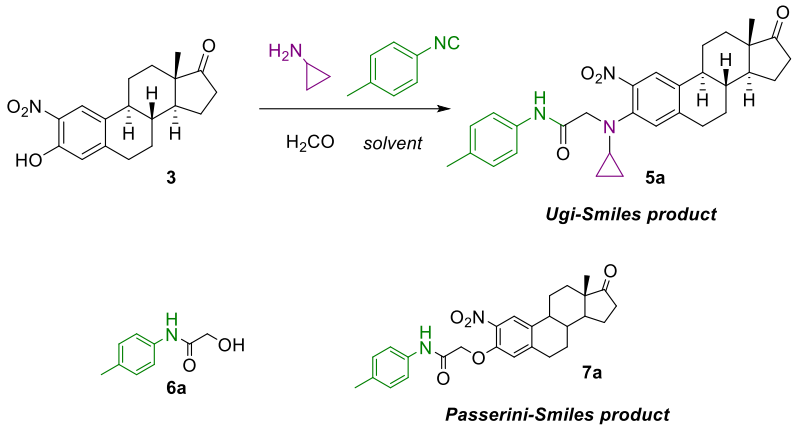


estrone

Scheme 1. Synthesis of nitroestrones. Reagent and conditions: a) HNO_3/AcOH 70°C to r.t., 18 h.

Then, in an exploratory assay, compound **3** was reacted with formaldehyde, cyclopropylamine and p-methylbenzyl isocyanide in different conditions. It is known that the Ugi-Smiles reaction can be performed both in protic and aprotic solvents with a wide range of polarity[22–24]. Nevertheless, the solubility of the steroidal component posed, in this case, an additional restriction on the solvent choice. On the other hand, temperatures ranging from 50°C - 80°C are usually needed as the Smiles rearrangement is an energy-demanding step. [6,22] When the reaction was carried out in toluene and ethanol no product was formed at a lower temperature (Table 1, entries 1 and 5) but the desired compound **5a** was observed in both solvents at 70°C (entries 2 and 6). The yield of **5a** could be improved using two equivalents of formaldehyde, amine and isocyanide, but with the concomitant formation of compound **6a** (entries 3 and 7), which is probably formed

by the reaction of the isocyanide with formaldehyde.[26] A further increase in the number of equivalents also led to the formation of compound **7a** (Scheme 2), generated by a competing Passerini-Smiles reaction, in which the amine component is not incorporated to the product.[22] Moreover, the same trend was observed when the reacion was carried out at higher temperatures (Table1, entry 9)



Scheme 2. Ugi-Smiles reaction on nitrosteroid **3** and structures of by-products.

The Table 1 summarizes the results of the different experiments and show that the best outcome was afforded when the reaction was performed in ethanol at 70°C, as the formation of by-products seems to be favored in toluene.

Table 1. Optimization of the reaction conditions.[a]

Entry	Solvent	Temperature (°C)	Number of equivalents[b]	5a[c]	6a[c]	7a[c]
1	ethanol	40	1	-	-	-
2	ethanol	70	1	36%	-	-
3	ethanol	70	2	60%	7%	-
4	ethanol	70	3	66%	11%	5%
5	toluene	40	1	-	-	-
6	toluene	70	1	15%	-	-
7	toluene	70	2	22%	3%	-
8	toluene	70	3	31%	23%	-
9	toluene	110	2	50%	26%	9%

a. Mean values from three independent experiments.
b. In relation to steroid.
b. Yield after chromatographic separation.

Having these improved conditions in hand, and in order to explore the scope of the reaction, compound **3** was treated with different amines, isocyanides and carbonyl compounds. As a general rule, when formaldehyde was used the reaction proceeded to give the desired products in moderate to good yields, incorporating a variety of fragments coming from alkyl amines both simple and having additional functional groups. However, less nucleophillic amines such as anilines, failed to give the reaction. On the other hand, the isocyano component proved to tolerate a broader structural diversity (Compounds **5b** – **5m**, Figure 2).

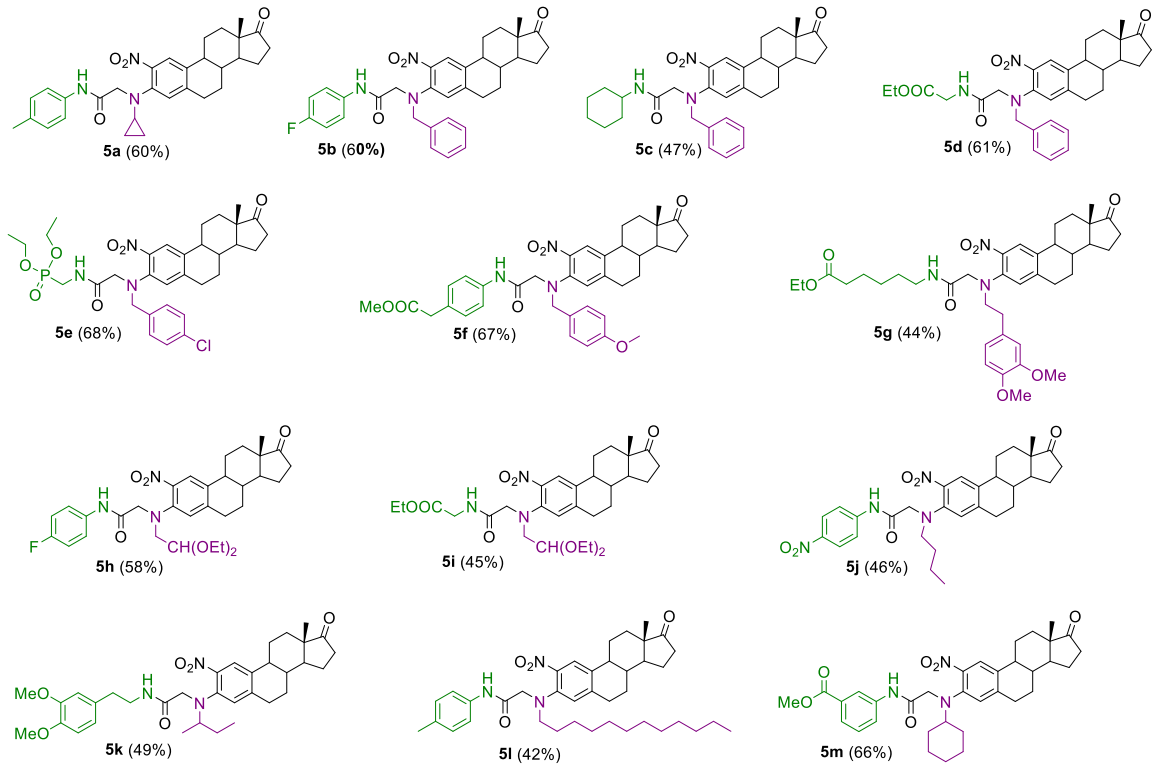


Figure 2. Structures of the compounds obtained from 2-nitroestrone **3** and different amines and isocyanides employing the optimized Ugi-Smiles reaction conditions. (Yields after chromatographic separation).

But probably the most striking result was to find that 4-nitroestrone (**4**) failed to give the desired Ugi-Smiles products when formaldehyde and the aforementioned amines and isocyanides were used. This unexpected fact suggests that the highly asymmetric environment generated by the steroidal skeleton might be responsible for the different reactivity between the two isomeric nitroestrones.

In order to gain more insight, we performed a preliminary theoretical study on the energetics of the process, where models for the intermediates and transitions states coming from each isomeric nitroestrone were constructed (for simplicity, methylamine and methylisocyanide were selected as components), and the energy barriers were calculated by DFT *ab initio* methods.

The calculations, performed at the M06-2X/6-31G* level using a continuum solvent model, suggest that the activation barrier for the Smiles rearrangement that connects the imidate intermediate **1** (Figure 1) with the corresponding product **2** is considerably higher for 4-nitroestrone than for 2-nitroestrone (28.1 vs 22.1 kcal/mol, respectively). A preliminary analysis shows that this difference could be attributable to the geometry of the nitro group relative to the aromatic ring in the transition state, that could affect the stabilization of the polar transition state.

Finally, we found that the reaction does not proceed when alternative carbonyl compounds such as alkyl and arylaldehydes or ketones were employed, in contrast to that observed when simpler nitrophenols were used

in the Ugi-Smiles condensation.[5] These results also suggest that the outcome of the Ugi-Smiles reaction is highly dependent on the influence of the steroidal polyciclic system.

In previous works we have shown that the structural diversification of the steroidal framework using isocyanide-based multicomponent reactions is a powerful strategy for the discovery of new bioactive entities.[27–29] In this sense, we studied the set of compounds described before in two *in vitro* phenotypic assays.

Firstly, our choice was a cell viability screening on the human colorectal adenocarcinoma HT29 cell line using the well-established MTT assay [30] after cell treatment with each compound during 48 h. Most of the steroids did not exert any effect below a 50 μM concentration (Figure 3a), except compound **5e**, which elicited a significant inhibition of cell viability with a IC_{50} value (the concentration needed to inhibit 50% of cell viability compared to that of the control) of 15 μM (Figure 3b). Consistently, this compound also showed an interesting antiproliferative effect on lung epithelial A549 cancer cells (IC_{50} = 18 μM).

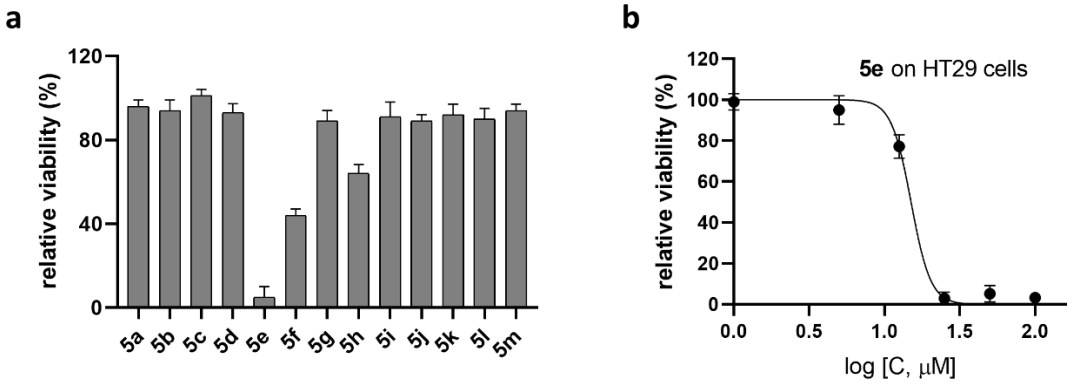


Figure 3. Antiproliferative effect of the Ugi-Smiles products on HT29 cancer cells.

In addition, we tested the synthetic compounds in an antiviral assay. To cover a broader spectrum of action, the assays were targeted against two mammalian viruses, HSV-1 and VSV, which belong to DNA and RNA viruses, respectively. The antiviral activity was expressed as the compound concentration required to reduce the virus yield by 50% (EC_{50}). The cytotoxic concentration required to reduce the Vero cell viability by 50 % (CC_{50}) was also calculated. This screening showed that compound **5b** has a potent antiviral effect (EC_{50} = 520 nM), with no cytotoxicity, against HSV-1 (KOS strain). Interestingly, this compound did not exert any effect on VSV multiplication.

In summary, in this work we have showed that the Ugi-Smiles reaction is a suitable synthetic tool for obtaining structurally diverse *N,N*-disubstituted 3-aminoestrones in a straightforward way when compared to previously reported methodologies. Our results highlight the scope and limitations of the reaction and

demonstrate that its outcome is highly influenced by the steroidal skeleton, an effect not observed when the Ugi-Smiles reaction is applied to more simple activated phenols. Finally, we found that some of the resulting compounds exert promising biological activities, such as antiproliferative and antiviral. To note, the simplicity of the synthetic approach might pave the way for an efficient hit-to-lead optimization for expanding this preliminary albeit promising results.

EXPERIMENTAL.

Synthesis of the compounds

General

All reagents were of analytical grade and were purchased from Sigma-Aldrich Chemical Co. Solvents were purchased from local suppliers and were distilled before use. Thin Layer Chromatography (TLC) were carried out using pre-coated plates of silica gel 60 F254from Merck and compounds were visualized by UV detection (254 nm) and sulfuric acid in ethanol stain. Purification of all compounds was carried out by column chromatographic using silica gel 60 0.040-0.063 mm from Merck. ESI-HRMS were measured on a Bruker micrOTOF-Q II. All NMR spectra were recorded on a Bruker AM-500 (500 MHz for ^{1}H and 125.1 MHz for ^{13}C). Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constant (J) values are quoted in Hz. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet) or combinations thereof. Structural determinations were confirmed by 2D NMR spectra (COSY, HSQC, HMBC and ROESY). In selected cases MS analysis are reported to further corroborate the structures.

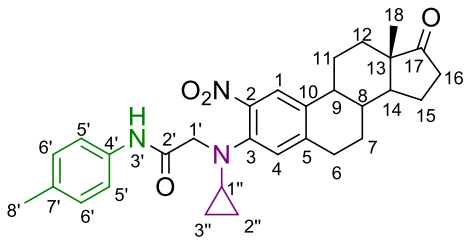
Starting materials.

Nitrosteroids **3** and **4** were synthesized from commercial estrone following the protocol of Tomson and Horwitz[21]. Ethylisocyano acetate, cyclohexyl isocyanide, and the amines employed were from commercial sources, whereas the rest of the isocyanides were synthetized from the corresponding amines following reported procedures[31].

General procedure for the Ugi-Smiles reaction.

The corresponding amine (typically 0.50 mmol) and formaldehyde (0.50 mmol, 88 µl, 37% aqueous solution) were dissolved in 0.3 mL of ethanol and stirred at room temperature for 1 hour. This mixture was dropped to a suspension of 2-nitroestrone **3** (0.25 mmol in 0.5 mL solvent of ethanol). Then, the isocyanide component (0.5 mmol) was added and the resulting reaction mixture was stirred at 70°C for 48 h. Finally, the reaction mixture was concentrated under vacuum to give a crude product which was purified by column chromatography to give the corresponding products **5a**-**5m**.

*Compound **5a***

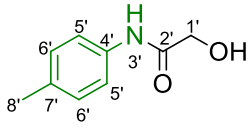


Purified by column chromatograph (ethyl acetate 10-50% gradient in petroleum ether). 42 mg (60% yield). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H, H-3'), 7.69 (s, 1H, H-1), 7.54 (d, 2H, *J = 8.5 Hz*, H-5') , 7.10 (d, 2H, *J = 8 Hz*, H-6') 6.82 (s, 1H, H-4), 4.22 (2d, 2H, *J = 17.5 Hz*, H-1'), 2.91 (m, 1H, H-1''), 2.87 (m, 2H, H-6), 2.51 (dd, 1H, *J = 19.5; 9 Hz*, H-16β), 2.38 (m, 1H, H-11β), 2.29 (s, 3H, H-8'), 2.21 (td, 1H, *J = 10; 3 Hz*, H-9α), 2.15 (dt, 1H, *J = 19.5, 9 Hz*, H-16α), 2.03 (m, 1H, H-15β), 2.01 (m, 1H, H-7α), 1.98 (m, 1H, H-12β), 1.61 (m, 1H, H-15α), 1.55 (m, 1H, H-8β), 1.54 (m, 1H, H-11α), 1.51 (m, 1H, H-14α), 1.46 (m, 1H, H-12α), 1.41 (m, 1H, H-7β), 0.91 (s, 3H, H-18), 0.69 (m, 2H, H-2''), 0.45 (m, 2H, H-3'').

^{13}C NMR (126 MHz, $CDCl_3$) δ 220.2 (C-17), 167.8 (C-2'), 143.6 (C-5), 140.2 (C-2), 140.0 (C-3), 135.4 (C-4'), 134.0 (C-10), 133.8 (C-7'), 129.4 (C-6'), 123.0 (C-1), 121.0 (C-4), 119.4 (C-5'), 56.6 (C-1'), 50.3 (C-14), 47.8 (C-13), 43.6 (C-9), 37.7 (C-8), 36.7 (C-1''), 35.8 (C-16), 31.3 (C-12), 29.5 (C-6), 26.0 (C-7), 25.5 (C-11), 21.5 (C-15), 20.8 (C-8'), 13.8 (C-18), 9.24 (C-2''), 9.21 (C-3''). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{30}H_{36}N_3O_4$: 502.2700, found: 502.2689.

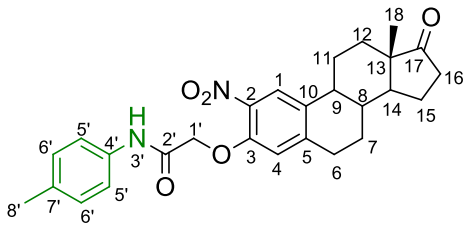
Further elution gave compounds **6a** and **7a** as main by-products.

*Compound **6a***



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, *J = 8.5 Hz*, 2H, H-5'), 7.07 (d, J = 8 Hz, 2H, H-6'), 4.05 (s, 2H, H-1'), 2.25 (s, 3H, H-8'). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8 (C-2'), 134.3 (C-4'), 134.2 (C-7'), 129.3 (C-6'), 119.8 (C-5'), 61.8 (C-1'), 20.6 (C-8').

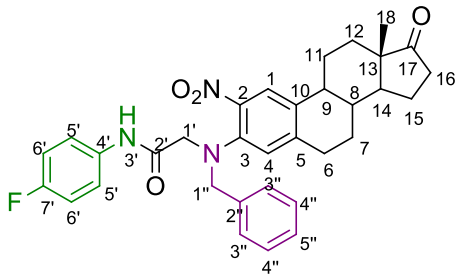
*Compound **7a***



Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H, H-3'), 8.03 (s, 1H, H-1), 7.60 (d, 2H, *J = 8.5 Hz*, H-5'), 7.16 (d, 2H, *J = 8 Hz*, H-6'), 6.79 (s, 1H, H-4), 4.70 (s, 2H, H-1'), 2.98 (m, 2H, H-6), 2.53 (dd, 1H, *J = 19; 8.5 Hz*,

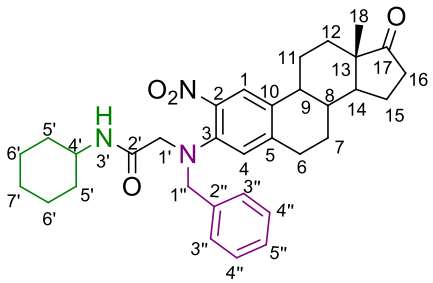
H-16β), 2.44 (m, 1H, H-11β), 2.33 (s, 3H, H-8'), 2.29 (m, 1H, H-9α), 2.17 (dt, 1H, J = 19.5; 9 Hz, H-16α), 2.09 (m, 1H, H-7α), 2.08 (m, 1H, H-15β), 2.01 (m, 1H, H-12β), 1.65 (m, 1H, H-15α), 1.57 (m, 1H, H-8β), 1.55 (m, 1H, H-11α), 1.53 (m, 1H, H-14α), 1.52 (m, 1H, H-12α), 1.50 (m, 1H, H-7β), 0.93 (s, 3H, H-18β). ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.0 (C-17), 164.6 (C-2'), 149.0 (C-3), 146.1 (C-5), 136.6 (C-2), 134.7 (C-4'), 134.4 (C-10), 134.3 (C-7'), 129.6 (C-6'), 124.0 (C-1), 119.6 (C-5'), 114.8 (C-4), 68.0 (C-1'), 50.2 (C-14), 47.8 (C-13), 43.5 (C-9), 37.7 (C-8), 35.8 (C-16), 31.3 (C-12), 29.8 (C-6), 25.9 (C-7), 25.7 (C-11), 21.5 (C-15), 20.9 (C-8'), 13.8 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{27}H_{31}N_2O_5$: 463.227, found: 463.229.

*Compound **5b***



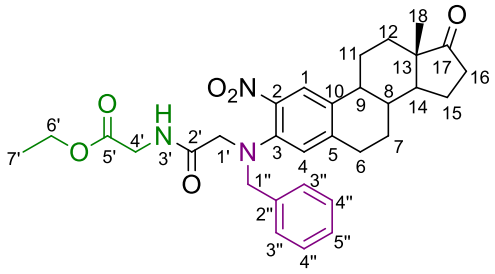
Purified by column chromatograph (ethyl acetate 20-50% gradient in petroleum ether). 51 mg (60% yield with traditional method, 21% with *one pot method*). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.57 (s, 1H, H-3') , 7.76 (s, 1H, H-1), 7.61 (m, 2H, H-5'), 7.29 (m, 2H, H-4''), 7.28 (m, 1H, H-5''), 7.12 (m, 2H, H-3''), 6.99 (m, 2H, H-6'), 6.94 (s, 1H, H-4), 4.10 (s, 2H, H-1''), 3.86 (2d, 2H, *J = 17 Hz*, H-1'), 2.90 (m, 2H, H-6), 2.52 (dd, 1H, *J = 19.5; 8.5 Hz*, H-16β), 2.39 (m, 1H, H-11β), 2.28 (td, 1H, *J = 11; 4.5 Hz*, H-9α), 2.16 (dt, 1H, *J = 19; 9 Hz*, H-16α), 2.053 (m, 1H, H-7α), 2.05 (m, 1H, H-15β), 2.00 (m, 1H, H-12β), 1.62 (m, 1H, H-15α), 1.58 (m, 1H, H-8β), 1.57 (m, 1H, H-11α), 1.51 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.45 (m, 1H, H-7β), 0.92 (s, 3H, H-18β). ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.0 (C-17), 167.7 (C-2'), 159.2 (d, $J_{C\text{-}F}$ *= 243.4 Hz*, C-7'), 144.0 (C-5), 142.7 (C-2), 141.3 (C-3), 137.0 (C-10), 135.3 (C-2''), 134.0 (d, $J_{C\text{-}F}$ *= 2.77 Hz*, C-4'), 128.9 (C-3''), 128.8 (C-4''), 128.2 (C-5''), 123.9 (C-4), 123.2 (C-1), 120.9 (d, $J_{C\text{-}F}$ *= 7.8 Hz*, C-5'), 115.6 (d, $J_{C\text{-}F}$ *= 22.4 Hz*, C-6'), 61.1 (C-1''), 55.2 (C-1'), 50.3 (C-14), 47.8 (C-13), 43.7 (C-9), 37.6 (C-8), 35.7 (C-16), 31.3 (C-12), 29.5 (C-6), 25.9 (C-7), 25.5 (C-11), 21.5 (C-15), 13.8 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{33}H_{35}FN_3O_4$: 556.2606, found: 556.2621.

*Compound **5c***



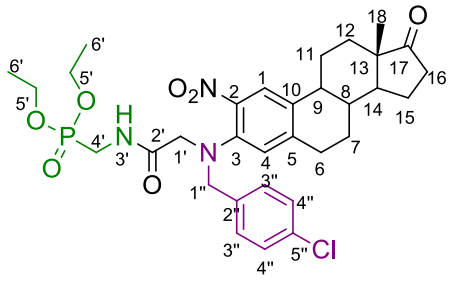
Purified by column chromatograph (ethyl acetate 10-40% gradient in petroleum ether). 30 mg (47% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 7.72 (s, 1H, H-1), 7.45 (d, *J* = 8 *Hz*, 1H, H-3'), 7.274 (m, 2H, H-4''), 7.27 (m, 1H, H-5''), 7.09 (dd, *J* =7.5; 2 *Hz*, 2H, H-3''), 6.87 (s, 1H, H-4), 4.05 (2d, *J =14 Hz*, 2H, H-1''), 3.70 (s, 2H, H-1'), 3.67 (m, 1H, H-4'), 2.88 (m, 2H, H-6), 2.51 (dd, *J = 19; 9 Hz*, 1H, H-16β), 2.38 (m, 1H, H-11β), 2.26 (td, *J = 11; 5 Hz,* 1H, H-9α), 2.15 (dt, *J = 19.5; 9 Hz*, 1H, H-16α), 2.06 (m, 1H, H-15β), 2.05 (m, 1H, H-7α), 1.99 (m, 1H, H-12β), 1.77 (m, 2H, H-5'), 1.65 (m, 1H, H-7'), 1.64 (m, 1H, H-15α), 1.59 (m, 1H, H-8β), 1.57 (m, 1H, H-11α), 1.56 (m, 4H, H-6'), 1.52 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.45 (m, 1H, H-7β), 1.30 (m, 1H, H-7'), 1.14 (m , 2H, H-5'), 0.92 (s, 3H, H-18β). ^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.12 (C-17), 168.3 (C-2'), 143.6 (C-5), 142.4 (C-3), 141.7 (C-2), 136.2 (C-10), 135.8 (C-2''), 128.8 (C-3''), 128.6 (C-4''), 128.0 (C-5''), 123.8 (C-4), 122.9 (C-1), 60.2 (C-1''), 55.2 (C-1'), 50.3 (C-14), 47.8 (C-4'), 43.7 (C-13), 43.68 (C-9), 37.7 (C-8), 35.8 (C-16), 32.8 (C-5'), 31.3 (C-12), 29.4 (C-6), 26.0 (C-7), 25.7 (C-6'), 25.6 (C-11), 24.7 (C-7'), 21.5 (C-15), 13.8 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{33}H_{42}N_3O_4$: 544.3170, found: 544.3158.

Compound ***5d***



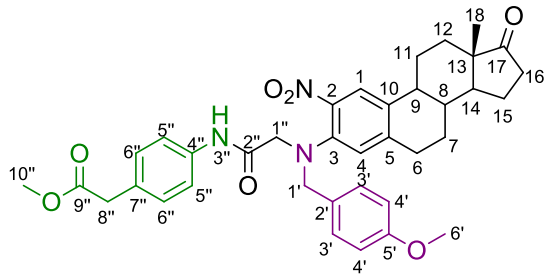
Purified by column chromatograph (ethyl acetate 30-50% gradient in petroleum ether). 46 mg (61% yield). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (t, *J = 6 Hz*, 1H, H-3'), 7.73 (s, 1H, H-1), 7.28 (m, 2H, H-4''), 7.26 (m, 1H, H-5''), 7.12 (m, 2H, H-3''), 6.87 (s, 1H, H-4), 4.18 (q, *J = 7 Hz*, 2H, H-6'), 4.13 (s, 2H, H-1''), 4.01 (dd, *J = 6; 2 Hz*, 2H, H-4'), 3.77 (s, 2H, H-1'), 2.87 (m, 2H, H-6), 2.51 (dd, 1H, *J* = 19.5; 9 Hz, H-16β), 2.38 (m, 1H, H-11β), 2.26 (td, *J = 11; 4.5 Hz*, 1H, H-9α), 2.15 (dt, *J = 19.5; 8.5 Hz*, 1H, H-16α), 2.06 (m, 1H, H-15β), 2.04 (m, 1H, H-7α), 1.99 (m, 1H, H-12β), 1.62 (m, 1H, H-15α), 1.58 (m, 1H, H-8β), 1.56 (m, 1H, H-11α), 1.49 (m, 1H, H-14α), 1.48 (m, 1H, H-12α), 1.45 (m, 1H, H-7β), 1.24 (t, *J = 7 Hz*, 3H, H-7'), 0.92 (s, 3H, H-18β). ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.1 (C-17), 170.1 (C-2'), 169.3 (C-5'), 143.6 (C-5), 142.4 (C-2), 141.7 (C-3), 136.3 (C-10), 135.7 (C-2''), 128.9 (C-3''), 128.6 (C-4''), 127.9 (C-5''), 123.9 (C-4), 123.1 (C-1), 61.3 (C-6'), 59.8 (C-1''), 55.1 (C-1'), 50.3 (C-14), 47.7 (C-13), 43.7 (C-9), 41.1 (C-4'), 37.6 (C-8), 35.7 (C-16), 31.3 (C-12), 29.4 (C-6), 25.9 (C-7), 25.5 (C-11), 21.5 (C-15), 14.1 (C-7'), 13.8 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{31}H_{38}N_3O_6$: 548.2755, found: 548.2774.

Compound ***5e***



Purified by column chromatograph (ethyl acetate). 111 mg (68% yield). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (s, 1H, H-1), 7.61 (td, 1H, *J = 6; 2.5 Hz*, H-3'), 7.23 (d, 2H, *J = 8.5 Hz*, H-4''), 7.05 (d, 2H, *J = 8 Hz*, H-3''), 6.85 (s, 1H, H-4), 4.08 (2dq, 4H, *J = 15; 7 Hz*, H-5'), 4.04 (s, 2H, H-1''), 3.71 (s, 2H, H-1'), 3.67 (2dd, 2H, *J = 12; 6 Hz*, H-4'), 2.86 (m, 2H, H-6), 2.50 (dd, 1H, J = *19.5; 8.5 Hz*, H-16β), 2.36 (m, 1H, H-11β), 2.24 (td; 1H, *J = 10.8; 4.2 Hz*, H-9α), 2.14 (dt, 1H, J = 19; 9 Hz, H-16α), 2.05 (m, 1H, H-15β), 2.04 (m, 1H, H-7α), 1.98 (m, 1H, H-12β), 1.61 (m, 1H, H-15α), 1.56 (m, 1H, H-8β), 1.54 (m, 1H, H-11α), 1.49 (m, 1H, H-14α), 1.47 (m, 1H, H-12α), 1.43 (m, 1H, H-7β), 1.27 (2t, 6H, *J = 7 Hz*, H-6'), 0.90 (s, 3H, H-18β). ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.0 (C-17), 169.2 (d, J_{CP} *= 4.9 Hz*, C-2'), 143.6 (C-5), 142.6 (C-2), 141.0 (C-3), 136.8 (C-10), 134.0 (C-2''), 133.8 (C-5''), 130.1 (C-3''), 128.8 (C-4''), 123.9 (C-4), 123.0 (C-1), 62.35 (d, J_{CP} *= 6.3 Hz*, C-5'), 59.0 (C-1''), 55.4 (C-1'), 50.2 (C-14), 47.7 (C-13), 43.7 (C-9), 37.5 (C-8), 35.7 (C-16), 34.4 (d, J_{CP} *= 156.2 Hz*, C-4'), 31.2 (C-12), 29.4 (C-6), 25.9 (C-7), 25.5 (C-11), 21.4 (C-15), 16.3 (d, J_{CP} *= 5.8 Hz*, C-6') 13.7 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{32}H_{42}ClN_3O_7P$: 646.2443, found: 646.2478.

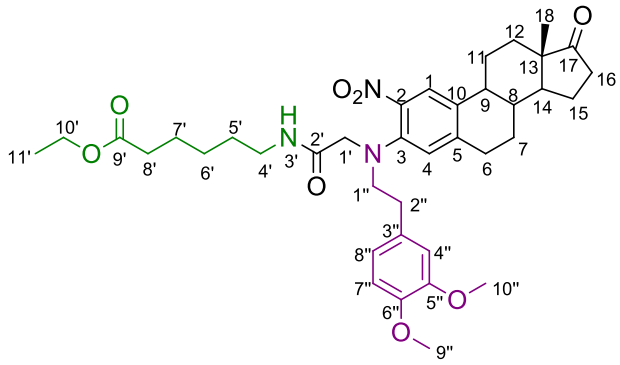
Compound ***5f***



Purified by column chromatograph (ethyl acetate 30-40% gradient in petroleum ether). 41 mg (67% yield). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.51 (s, 1H, H-3''), 7.73 (s, 1H, H-1), 7.60 (d, 2H, *J = 8.56 Hz*, H-5''), 7.2 (d, 2H, *J = 8.56 Hz*, H-6''), 7.03 (d, 2H, *J = 8.68 Hz*, H-3'), 6.92 (s, 1H, H-4), 6.81 (d, 2H, *J = 8.64 Hz*, H-4'), 4.03 (s, 2H, H-1'), 3.83 (2d, 2H, *J = 17,5 Hz*, H-1''), 3.77 (s, 3H, H-6'), 3.66 (s, 3H, H-10''), 3.57 (s, 2H, H-8''), 2.89 (m, 2H, H-6), 2.51 (dd, 1H, *J = 19; 9 Hz*, H-16β) 2.38 (m, 1H, H-11β) , 2.27 (td, 1H, *J = 11; 5 Hz*, H-9α), 2.15 (dt, 1H, *J = 19; 9 Hz*, H-16α), 2.06 (m, 1H, H-15β), 2.04 (m, 1H, H-7α), 2.00 (m, 1H, H-12β), 1.62 (m, 1H, H-15α), 1.59 (m, 1H, H-8β), 1.57 (m, 1H, H-11α), 1.5 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.45 (m. 1H, H-7β), 0.91 (s, 3H, H-18β). ^{13}C NMR (500MHz, $CDCl_3$) δ (ppm) 220.1 (C-17), 172.0 (C-9''), 167.8 (C-2''), 159.4 (C-5'),

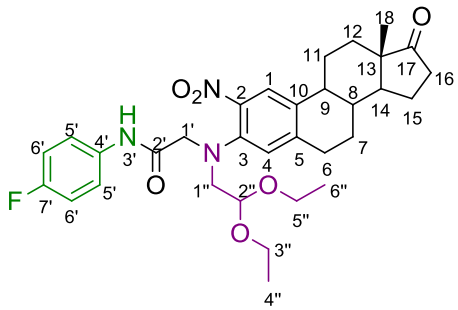
143.9 (C-5), 142.7 (C2), 141.1 (C-3), 137.0 (C-4''), 136.8 (C-10), 130.2 (C-3'), 129.7 (C-6''), 129.6 (C-7''), 127.3 (C-2'), 123.6 (C-4), 123.1 (C-1), 119.4 (C-5''), 114.1 (C-4'), 60.6 (C-1'), 55.2 (C-6'), 54.8 (C-1''), 52.0 (C-10''), 50.2 (C-14), 47.8 (C-13), 43.7 (C-9), 40.6 (C-8''), 37.5 (C-8), 35.7 (C-16), 31.3 (C-12), 29.4 (C-6), 25.9 (C-7), 25.5 (C-11), 21.5 (C-15), 13.8 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{37}H_{42}N_3O_7$: 640.3017,found: 640.3015.

Compound ***5g***



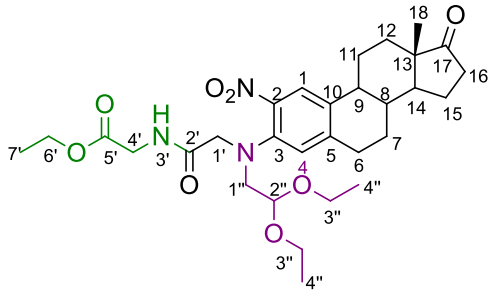
Purified by column chromatograph (ethyl acetate 30-80% gradient in petroleum ether). 62 mg (44% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 7.64 (s, 1H, H-1), 7.35 (t, *J = 5.5 Hz*, 1H, H-3'), 6.73 (s, 1H, H-4), 6.70 (d, *J = 8.5 Hz*, 1H, H-7''), 6.56 (dd, *J = 8 ;2 Hz*, 1H, H-8''), 6.45 (d, *J = 2 Hz*, 1H, H-4''), 4.08 (q, *J = 7 Hz*, 2H, H-10'), 3.81 (s, 3H, H-9''), 3.80 (s, 2H, H-1'), 3.77 (s, 3H, H-10''), 3.20 (t, *J = 7 Hz*; 2H, H-1''), 3.15 (m, 2H, H-4'), 2.81 (m, 2H, H-6), 2.65 (dd coupled, *J = 8; 6.5 Hz*; 2H, H-2''), 2.51 (dd, *J = 19; 8.5 Hz*, 1H, H-16β), 2.35 (m, 1H, H-11β), 2.22 (m, 1H, H-9α), 2.20 (t, *J = 8 Hz*, 2H, H-8'), 2.14 (dt, *J = 19; 8.5 Hz*, 1H, H-16α), 2.05 (m, 1H, H-15β), 2.02 (m, 1H, H-7α), 1.98 (m, 1H, H-12β), 1.62 (m, 1H, H-15α), 1.56 (m, 1H, H-8β), 1.55 (m, 1H, H-11α), 1.543 (q, *J = 7.5 Hz*, 2H, H-7'), 1.49 (m, 1H, H-14α), 1.48 (m, 1H, H-12α), 1.42 (m, 1H, H-7β), 1.41 (q, *J = 7.5 Hz*, 2H, H-5'), 1.22 (t, *J = 7.5 Hz*, 3H, H-11'), 1.21 (m, 2H, H-6'), 0.92 (s, 3H, H-18β). ^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.1 (C-17), 173.4 (C-9'), 169.5 (C-2'), 148.8 (C-5''), 147.5 (C-6''), 143.2 (C-5), 142.3 (C-2), 141.0 (C-3), 135.7 (C-10), 130.8 (C-3''), 123.4 (C-4), 122.7 (C-1), 120.7 (C-8''), 111.3 (C-4''), 111.0 (C-7''), 60.1 (C-10'), 56.7 (C-1''), 55.9 (C-1'), 55.8 (C-10''), 55.6 (C-9''), 50.2 (C-14), 47.8 (C-13), 43.6 (C-9), 38.9 (C-4'), 37.6 (C-8), 35.7 (C-16), 34.1 (C-8'), 33.3 (C-2''), 31.3 (C-12), 29.3 (C-6), 28.9 (C-5'), 26.2 (C-6'), 25.9 (C-7), 25.5 (C-11), 24.5 (C-7'), 21.5 (C-15), 14.2 (C-11'), 13.7 (C-18).

Compound **5h**



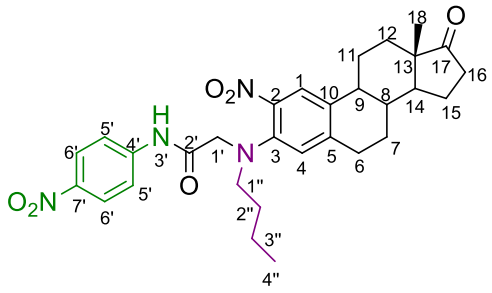
Purified by column chromatograph (ethyl acetate 30-50% gradient in petroleum ether). 43 mg (58% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 9.60 (s, 1H, H-3'), 7.72 (s, 1H, H-1), 7.63 (m, 2H, H-5'), 7.07 (s, 1H, H-4), 6.99 (m , 2H, H-6'), 4.46 (t, 1H, *J = 5 Hz*, H-2''), 4.09 (2d, 2H, *J = 17.5 Hz*, H-1'), 3.57 and 3.40 (2dq, 2H, *J = 7 Hz*, H-3'' and H-5''), 3.15 (d, 2H, *J = 5 Hz*, H-1''), 2.92 (m, 2H, H-6), 2.52 (dd, 1H, *J = 19.5; 8.5 Hz*, H-16β), 2.38 (m, 1H, H-11β), 2.27 (td, 1H, *J = 11; 4 Hz*, H-9α), 2.16 (dt, 1H, *J = 19.5; 8.5 Hz*, H-16α), 2.07 (m, 1H, H-7α), 2.06 (m, 1H, H-15β), 1.99 (m, 1H, H-12β), 1.65 (m, 1H, H-15α), 1.58 (m, 1H, H-8β), 1.57 (m, 1H, H-11α), 1.52 (m, 1H, H-14α), 1.51 (m, 1H, H-12α), 1.47 (m, 1H, H-7β), 1.13 and 1.12 (t, 3H, *J = 7 Hz*, H-4'' and H-6''), 0.91 (s, 3H, H-18β). ^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.0 (C-17), 168.3 (C-2'), 159.2 (d, *J = 243.2 Hz*, C-7'), 143.9 (C-5), 142.7 (C-2), 141.2 (C-3), 136.9 (C-10), 134.1 (d, *J = 2.77 Hz*, C-4'), 124.3 (C-4), 123.0 (C-1), 121.0 (d, *J = 7.81 Hz*, C-5'), 115.5 (d, *J = 22.3 Hz*, C-6'), 100.8 (C-2''), 62.73 and 62.72 (C-3''and C-5''), 58.1 (C-1''), 58.0 (C-1'), 50.3 (C-14), 47.8 (C-13), 43.7 (C-9), 37.6 (C-8), 35.7 (C-16), 31.3 (C-12), 29.5 (C-6), 25.9 (C-7), 25.5 (C-11), 21.5 (C-15), 15.24 and 15.22 (C-4'' and C-6''), 13.8 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{32}H_{41}FN_3O_6$: 582.2974, found: 582.2985.

*Compound **5i***



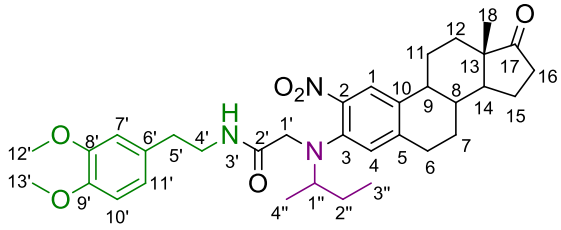
Purified by column chromatograph (ethyl acetate 30-80% gradient in petroleum ether). 67 mg (45% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 8.04 (t, *J = 6 Hz*, 1H, H-3'), 7.69 (s, 1H, H-1), 7.03 (s, 1H, H-4), 4.44 (t, *J = 5.5 Hz*, 1H, H-2''), 4.16 (q, *J = 7.2 Hz*, 2H, H-6'), 4.02 (d, *J= 6 Hz*, 2H, H-4'), 3.98 (s, 2H, H-1'), 3.55 (m, 2H, H-3''), 3.39 (m, 2H, H-3''), 3.15 (d, *J =5.5 Hz*, 2H, H-1''), 2.90 (m, 2H, H-6), 2.50 (dd, *J = 19 ;8.5 Hz*, 1H, H-16β), 2.37 (m, 1H, H-11β), 2.25 (td, *J = 10.5; 4.5 Hz*, 1H, H-9α), 2.14 (dt, *J = 19; 9 Hz,* 1H, H-16α), 2.05 (m, 1H, H-15β), 2.04 (m, 1H, H-7α), 1.98 (m, 1H, H-12β), 1.62 (m, 1H, H-15α), 1.57 (m, 1H, H-8β), 1.54 (m, 1H, H-11α), 1.51 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.44 (m, 1H, H-7β), 1.24 (t, *J* =7.17 Hz, 3H, H-7'), 1.11 (t, *J* = 7.03 Hz, 3H, H-4''), 1.10 (t, *J* = 7.04 Hz, 3H, H-4''), 0.90 (s, 3H, H-18β). ^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.1 (C-17), 170.6 (C-2'), 169.3 (C-5'), 143.5 (C-5), 142.6 (C-2), 141.5 (C-3), 136.4 (C-10), 124.5 (C-4), 122.8 (C-1), 100.8 (C-2''), 62.58 (C-3''), 62.56 (C-3''), 61.2 (C-6'), 57.6 (C-1'), 57.2 (C-1''), 50.2 (C-14), 47.8 (C-13), 43.7 (C-9), 41.1 (C-4'), 37.6 (C-8), 35.7 (C-16), 31.3 (C-12), 29.4 (C-6), 26.0 (C-7), 25.5 (C-11), 21.5 (C-15), 15.19 (C-4''), 15.18 (C-4''), 14.1 (C-7'), 13.7 (C-18). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{30}H_{44}N_3O_8$: 574.3123, found: 574.3099.

Compound ***5j***



Purified by column chromatograph (ethyl acetate 5-20% gradient in petroleum ether). 57 mg (46% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 10.05 (s, 1H, H-3'), 8.19 (d, *J = 9 Hz*, 2H, H-6'), 7.86 (d, *J = 9 Hz*, 2H, H-5'), 7.74 (s, 1H, H-1), 7.00 (s, 1H, H-4), 3.98 (2d, *J = 17 Hz*, 2H, H-1'), 2.97 (m, 2H, H-1''), 2.93 (m, 2H, H-6), 2.52 (dd, *J =19.5 ;8.5 Hz*, 1H, H-16β), 2.39 (m, 1H, H-11β) , 2.27 (td, *J =11; 4 Hz*, 1H, H-9α), 2.15 (dt, *J = 19; 9 Hz*, 1H, H-16α), 2.07 (m, 1H, H-15β), 2.06 (m, 1H, H-7α), 2.00 (m, 1H, H-12β), 1.63 (m, 1H, H-15α), 1.59 (m, 1H, H-8β), 1.57 (m, 1H, H-11α), 1.50 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.48 (m, 1H, H-7β), 1.41 (m, 2H, H-2''), 1.21 (sextuplet, *J = 7.5 Hz*, 2H, H-3'') 0.91 (s, 3H, H-18β), 0.84 (t, *J = 7.5 Hz*, 3H, H-4'').^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.0 (C-17), 169.2 (C-2'), 144.2 (C-5), 143.7 (C-4'), 143.5 (C-7'), 142.9 (C-2), 141.1 (C-3), 137.1 (C-10), 125.0 (C-6'), 124.1 (C-4), 123.2(C-1), 118.9 (C-5'), 56.73 (C-1'), 56.72 (C-1''), 50.2 (C-14), 47.8 (C-13), 43.7 (C-9), 37.6 (C-8), 35.7 (C-16), 31.3 (C-12), 29.5 (C-6), 28.8 (C-2''), 25.9 (C-7), 25.5 (C-11), 21.5 (C-15), 20.0 (C-3''), 13.8 (C-18), 13.7 (C-4'').

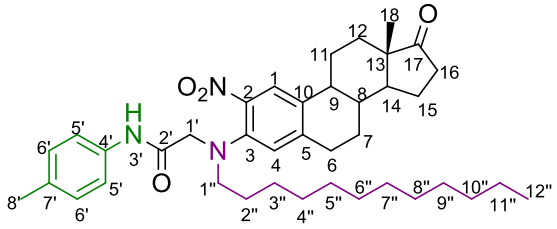
Compound ***5k***



Purified by column chromatograph (ethyl acetate 50-100% gradient in petroleum ether). 47 mg (49% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 7.79 (t, 1H, *J = 7.5 Hz*, H-3'), 7.56 (s, 1H, H-1), 6.93 (d, 1H, *J = 2.5 Hz*, H-4), 6.70 (d, 1H, *J = 8 Hz*, H-10'), 6.64 (s, 1H, H-7'), 6.63 (dd, 1H, *J = 8; 2.5 Hz*, H-11'), 3.83 (s, 1H, H-13'), 3.81 (s, 1H, H-12'), 3.74 (2s, 2H, H-1'), 3.4 (2dtd, 2H, *J = 13.5; 8; 7.5 Hz*, H-4'), 2.89 (m, 2H, H-6) , 2.79 (m, 1H, H-1''), 2.69 (ta, 2H, *J = 8 Hz*, H-5'), 2.51 (dd, 1H, *J = 19; 8.5 Hz*, H-16β), 2.35 (m, 1H, H-11β), 2.26 (td, 1H, *J = 10.5; 4.5 Hz*, H-9α), 2.15 (dt, 1H, *J = 19; 8.5 Hz*, H-16α), 2.06 (m, 1H, H-15β), 2.04 (m, 1H, H-7α), 2.00 (m, 1H, H-12β), 1.62 (m, 1H, H-15α), 1.59 (m, 1H, H-8β), 1.57 (m, 1H, H-11α), 1.53 (m, 1H, H-2''), 1.5 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.45 (m, 1H, H-7β), 1.28 (m, 1H, H-2''), 0.98 (dd, 3H, *J = 13.5; 7 Hz,* H-4''), 0.92 (s, 3H, H-18β), 0.76 (t, 3H, *J = 7 Hz*, H-3''). ^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.2 (C-17), 170.6 (C-2'), 148.8 (C-8'), 147.4 (C-9'), 142.9 (C-2), 142.9 (C-5), 141.0 (C-3), 135.7 (C-10), 131.2 (C-6'),

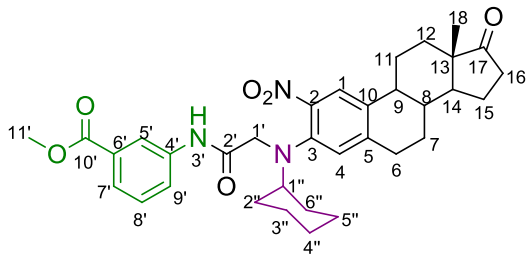
123.1 (C-4), 122.9 (C-1), 120.4 (C-11'), 111.6 (C-7'), 111.2 (C-10'), 63.3 (C-1''), 55.9 (C-13'), 55.7 (C-12'), 50.3 (C-14), 47.9 (C-13), 47.8 (C-1'), 43.6 (C-9), 40.2 (C-4'), 37.6 (C-8), 35.8 (C-16), 34.8 (C-5'), 31.3 (C-12), 29.4 (C-6), 27.2 (C-2''), 26.0 (C-7), 25.5 (C-11), 21.5 (C-15), 16.04 (C-4''), 13.8 (C-18), 11.3 (C-3''). HRMS (ESI) m/z calculated for $(M+H)^+$ $C_{34}H_{46}N_3O_6$: 592.3381, found: 592.3365.

*Compound **5l***



Purified by column chromatograph (ethyl acetate 10-30% gradient in petroleum ether). 52 mg (42% yield). Pale yellow oil. ^{1}H NMR (Mayor conformer, 500 MHz, $CDCl_3$) δ 9.47 (s, 1H, H-3'), 7.71 (s, 1H, H-1), 7.55 (d, *J = 8.5 Hz*, 2H, H-5'), 7.10 (d, *J = 8 Hz*, 2H, H-6'), 6.98 (s, 1H, H-4), 3.95 (2d coupled, *J = 17 Hz*, 2H, H-1'), 2.96 (m, 2H, H-1''), 2.92 (m, 2H, H-6) , 2.51 (dd, *J = 18.5; 8.75 Hz*, 1H, H-16β), 2.37 (m, 1H, H-11β), 2.29 (s, 3H, H-8'), 2.25 (td*, J = 11; 4.5 Hz*, 1H, H-9α), 2.15 (dt*, J = 19.5; 9 Hz*, 1H, H-16α), 2.06 (m, 1H, H-15β), 2.05 (m, 1H, H-7α), 1.99 (m, 1H, H-12β), 1.63 (m, 1H, H-15α), 1.58 (m, 1H, H-8β), 1.56 (m, 1H, H-11α), 1.50 (m, 1H, H-14α), 1.49 (m, 1H, H-12α), 1.45 (m, 1H, H-7β), 1.43 (m, 2H, H-2''), 1.28 (m, 2H, H-11''), 1.23 (m, 2H, H-10''), 1.17 (m, 2H, H-3''), 1.25-1.15 (m, 12H, H-4'' to H-9''), 0.91 (s, 3H, H-18β), 0.87 (t broad, *J = 7 Hz*, 3H, H-12''). ^{13}C NMR (Mayor conformer, 126 MHz, $CDCl_3$) δ 220.1 (C-17), 168.0 (C-2'), 143.7 (C-5), 142.7 (C-2), 141.3 (C-3), 136.3 (C-10), 135.4 (C-4'), 133.6 (C-7'), 129.4 (C-6'), 123.6 (C-4), 123.1 (C-1), 119.2 (C-5'), 56.7 (C-1''), 56.4 (C-1'), 50.3 (C-14), 47.8 (C-13), 43.7 (C-9), 37.6 (C-8), 35.7 (C-16), 31.9 (C-10''), 31.3 (C-12), 29.6-29.2 (C4'' to C-9'') , 29.5 (C-6), 26.9 (C-2''), 26.8 (C-3''), 26.0 (C-7), 25.5 (C-11), 22.7 (C-11''), 21.5 (C-15), 20.8 (C-8'), 14.1 (C-12''), 13.7 (C-18).

*Compound **5m***



Purified by column chromatograph (ethyl acetate 20-30% gradient in petroleum ether). 93 mg (66% yield). Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.93 (s, 1H, H-3'), 8.32 (t, 1H, *J = 2 Hz*, H-5'), 7.85 (ddd, 1H, *J = 8;*

2; 1 Hz, H-9'), 7.75 (dt *broad* , 1H, *J= 8; 2; 1 Hz*, H-7') , 7.68 (s, 1H, H-1), 7.37 (t *broad*, 1H, *J = 8 Hz*, H-8'), 7.00 (s, 1H, H-4), 3.97 (s, 2H, H-1'), 3.90 (s 3H, H-11'), 2.91 (m, 2H, H-6), 2.77 (tt, 1H, *J = 12; 3.5 Hz*, H-1''), 2.51 (dd, 1H, *J = 19.5; 9 Hz*, H-16β), 2.36 (m, 1H, H-11β), 2.25 (td, 1H, *J = 11; 4.5 Hz*, H-9α), 2.15 (dt, 1H, *J = 19; 9 Hz*, H-16α), 2.05 (m, 1H, H-15β), 2.04 (m, 1H, H-7α), 1.98 (m, 1H, H-12β), 1.85 (t *broad*, 2H, *J = 11.5 Hz*, H-6''), 1.75 (d, 2H, *J = 13.5 Hz*, H-5''), 1.61 (m, 1H, H-15α), 1.58 (m, 1H, H-11α), 1.58 (m, 1H, H-8β), 1.52 (m, 1H, H-4''), 1.50 (m, 1H, H-14α), 1.48 (m, 1H, H-12α), 1.45 (m, 1H, H-7β), 1.27 (m, 2H, H-2''), 1.17 (m, 2H, H-3''), 1.03 (qt, 1H, *J = 13; 3.5 Hz*, H-4''), 0.90 (s, 3H, H-18β). ^{13}C NMR (126 MHz, $CDCl_3$) δ 220.1 (C-17), 169.5 (C-2'), 166.8 (C-10'), 143.6 (C-2), 143.4 (C-5), 140.8 (C-3), 138.2 (C-4'), 136.8 (C-10), 130.9 (C-6'), 128.9 (C-8'), 125.2 (C-7'), 124.7 (C-4), 123.7 (C-9'), 123.1 (C-1), 120.2 (C-5'), 66.1 (C-1''), 52.2 (C-11'), 51.1 (C-1'), 50.2 (C-14), 47.8 (C-13), 43.7 (C-9), 37.6 (C-8), 35.7 (C-16), 31.3 (C-12), 29.8 (C-2''), 29.5 (C-6''), 29.48 (C-6), 25.9 (C-7), 25.7 (C-3'' and C-5''), 25.5 (C-4''), 25.4 (C-11), 21.5 (C-15), 13.8 (C-18).

Computational methods

We have performed DFT calculations with Gaussian 09[32] using the M06-2X[33] functionals. An ultrafine integration grid was used for all calculations. Optimizations were carried out with the 6-31G(d) basis set in ethanol with the IEFPCM[34] solvent model. Single point energies[35] were obtained for the optimized structures using the M06-2X functional with the polarized, triple-zeta valence quality def2-TZVPP basis set.[36]

Biological assays

Antiproliferative assay

HT29 and A549 cells were all obtained from ATCC (Rockville, MD, USA). Cells were seeded in DMEM medium (Gibco, Grand Island, NY, USA), which was supplemented with 10% fetal bovine serum (Gibco) and 50 µg/ml gentamicin. Culture cells were maintained in 5% CO_2 atmosphere at 37 °C.

Cell viability was determined by colourimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich Co) assay. Cells were seeded in 96-well plates at a density of $1x10^4$ cells/well and solutions containing different concentrations of the tested compound were added. Each assay was done in triplicate. After 72 h of incubation, MTT solution (5 mg/mL in distilled water) was added to cells in the culture medium and plates were incubatedfor 2 h at 37 °C. Then, the produced formazan was solubilised by the addition of 0.2 mL ethanol and absorbance values were immediately measured using an ELISA plate reader (Eurogenetics MPR-A 4i) at a test wavelength of 570 nm and a reference wavelength of 630 nm. Results for each treated cell culture were normalised as a percentage of absorbance with respect to untreated controls. The standard deviations (SDs) of the normalised data were calculated from the SDs of treated cells and controls and the mean values of the corresponding absorbances (using data from three independent experiments).

Antiviral assay.

Vero cells grown in 96-well tissue culture plates were infected with HSV-1 (strains KOS or B2006) or VSV. After 1 h adsorption at 37°C the inoculum was removed and medium containing different concentrations of compounds **4a-j** was added, by triplicate. The plates were incubated at 37°C in a 5% CO2 atmosphere until 100% cell death was observed microscopically in untreated infected control cells. Supernatants corresponding to those triplicates were harvested after cell disruption by three cycles of freezing and thawing and pooled. Virus yields were titrated by plaque assay. For comparative purposes, ACV was tested as positive control against HSV-1.

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