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Aza-annulation on the 16-dehydropregnenolone, via tandem intermolecular Aldol process and intramolecular Michael addition

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ABSTRACT

16-Dehydropregnenolone undergoes a smooth annulation with propan-1-amine and aromatic aldehydes. Several amine derivatives of 16- dehydropregnenolone were synthesized and evaluated as inhibitors of DPP-IV. The structures of compounds were confirmed by ^{1}H, ^{13}C, NMR and mass spectral analysis. Among 17 compounds evaluated only five compounds **1**, **9**, **13**, **15** and **16** demonstrated significant inhibition of DPP. This study suggest that introduction of appropriate substituents in the 16-dehydropregnenolone plays an important role in DPP-IV inhibitory activity.

The protein encoded by the DPP-IV gene is an antigenic enzyme expressed on the surface of most cell types and is associated with immune regulation, signal transduction and apoptosis. It is an intrinsic membrane glycoprotein and a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP-IV plays a major role in glucose metabolism. It is responsible for the degradation of incretins such as glucagon-like-peptide-1 (GLP-1) and GIP.[1] By inactivating incretins, DPP-IV prevents the incretins from having significant effects on insulin secretion and blood glucose levels. They can be used to treat diabetes mellitus type 2.

Pregnenolone is a major hormone mainly present in human nerve tissues.[2] Recent studies indicated that derivatives of pregnenolone have many profound activities, such as anti-inflammatory,[3,4] anti-asthamatic,[5] cytotoxic,[6] anti-feedant,[7] lipid lowering[8] and anti-viral[9], neurosteroids[10] and as inhibitors of testosterone 5α reductase[11] which helps in the treatment of androgen sensitive prostate cancer in men.[12] Some pregnanes are reported binding to the cardiac glycoside recognition site on Na^{+}, K^{+}-ATPase and inhibit the enzyme (the sodium pump) in membranes, cells and tissues.[13] Steroids of the 5α or 5β-pregnane or androstane series containing a 3α-hydroxy group and a hydrogen bond acceptor group attached with the β-configuration at C-17 are known to have anaesthetic activity.[14] 16-Dehydropregnenolone acetate (16-DPA) finds increasing application as a versatile scaffold and building block for different steroidal drugs for it is an ideal platform for preparation of dexamethasone, β-methasone, 5α-reductase inhibitor, and related other steroidal pharmacophores.[15]

Numerous modifications to the steroid nucleus have been made in order to study the SAR of bioactive steroids, including substitutions at the 3β-, 11-, 17-, or 21-positions. The presence of substituent on C(16) and C(18) of steroids has been shown to have a profound effect on physiological activity. As a result, recently there has been considerable interest in the preparation of steroids substituted at these positions.[16,17] These observations inspired several groups to synthesize various medicinally important derivatives of pregnane with the intention to either improve biochemical and pharmacokinetic characteristics of the parent drug or to obtain compounds containing essential elements of the parent substance but having novel properties and/or affecting novel molecular targets.[18–24] Some example of the formation, in steroids, of a new ring fused to ring D in positions 16 and 17 have reported in the literature.[25] Steroid alkaloids are also of interest because of their broad spectrum of biological activity.[26] They form a large group of molecules where a nitrogen atom is integrated into a ring or in a substituent.

The isolation and synthesis of many biologically important steroids with modified side chains,[27] such as ecdysones,[28] metabolite of vitamin D3,[29] brassinosteroids,[30] squalamine,[31] OSW-1,[32] contignasterol,[33] and marine sterols,[34] have stimulated much interest to introduce such modified side chains into readily available steroids.

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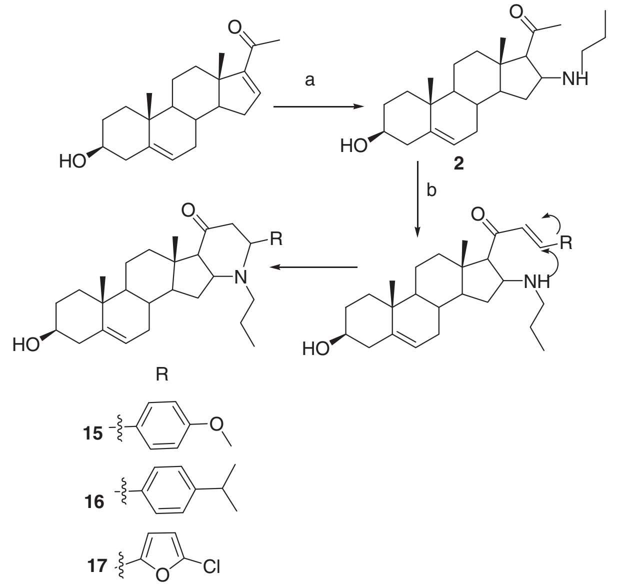
In the present study, we have fused substituted piperidine to ring D by applying Aldol condensation and Michael addition reaction. Steroids with a new pentacyclic ring system have been synthesized. This Letter reports structure determination of these pentacyclic compounds and also proposes a mechanism for formation of six member ring. As far as we know the reaction reported in this manuscript represent a new type of cyclization in the field of steroids.

The syntheses of amine analogues of pregnenolone were accomplished by subjecting the 16-dehydropregnenolone using various aromatic and aliphatic amines by employing Michael addition reaction at 45 °C for 4 h to afford amine derivative of pregnenolone (**2**–**12**) in very good yield (Scheme 1). The ^{1}H NMR spectrum not only conclusively proved the structure of compounds but it also helped in ascertaining the orientation of side chain at C-16. A well-defined doublet at d 2.58 was attributed to the methine proton at C-17.The magnitude of the coupling constant of this doublet, J = 6.4 Hz confirms the orientation of this side chain at C-16 to be α.[35]

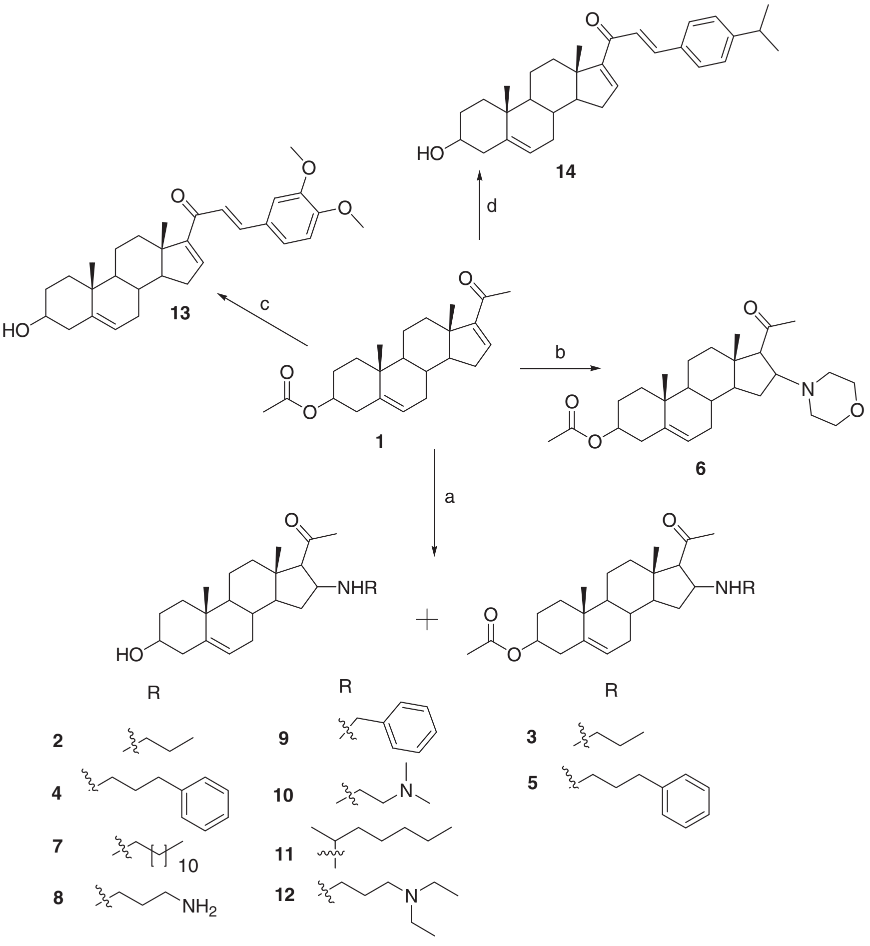
The amine **2** was subjected to Aldol condensation reaction with various aldehydes in ethanol using KOH as a base at room temperature to afford condensed compounds which on intramolecular Michael addition reaction afforded compound **15**, **16** and **17** (Scheme 2).

We have synthesized some more derivative of 16-dehydropregnenolone by changing the substituent at C-16 and C-17 positions. Compounds **13** and **14** were synthesized using base catalyzed Aldol condensation of acetylated 16-dehydropregnenolone with 3, 4-dimethoxybenzaldehyde and 4-isopropylbenzeldehyde respectively (Scheme 1).



Scheme 2. Synthesis of Aza derivatives of 16-dehydropregnenolone. Reagents and conditions: (a) propyl amine, 45 °C, 4 h; (b) RCHO, EtOH, KOH, rt, 24 h.

All the compounds were fully characterized and evaluated for DPP-IV inhibitory activity. The test compounds on DPP-IV enzyme



Scheme 1. Synthesis of different amine derivatives of acetylated 16-dehydropregnenolone. Reagents and conditions: (a) RNH$_2$, 45 °C, 4 h; (b) morpholine, 45 °C, 4 h; (c) 3,4-dimethoxy benzaldehyde, EtOH, KOH, rt, 24 h; (d) 4-isopropyl benzaldehyde, EtOH, KOH, rt, 24 h.

activity was studied by incubating these with human recombinant DPP-IV and determining the residual DPP-IV activity using chromogenic substrate using DPP-IV drug discovery kit-BML-AK 499 from Enzo Life Sciences, USA. The principle of the assay in the kit was essentially as described in the literature.[36,37] The kit provided recombinant soluble human DPP-IV enzyme, chromogenic substrate (H-Gly-Pro-AMC, K_m 114 μM), assay buffer, DPP-IV inhibitor and Calibration Standard. Cleavage of the substrate by enzyme DPP-IV increases absorbance at 405 nm. The reaction was carried out in 96 well flat bottomed microtiter plates by the addition of assay buffer, enzyme DPP-IV and diluted chromogenic substrate (H-Gly-Pro-pNA). The solutions of test substances were prepared in DMSO and of which 10 μl solution was added in each reaction well to achieve 10 μM concentration in each well. The plate was incubated at 37 °C for 10 min. After incubation the plate was read continuously at 405 nm in a double beam spectrophotometer. As cleavage of chromogenic substrate (H-Gly-Pro-pNA; K_m = 114 μM) increases absorbance at 405 nm. The percentage inhibition by test compounds on DPP-IV enzyme was calculated based on activity in the control tube as 100% from three independent set of experiments. The concentration of DMSO in the test well (1.0%) had no demonstrable effect on DPP-IV enzyme activity.

Out of 17 compounds tested, five compounds **1**, **9**, **13**, **15** and **16** were found to have significant effect on DPP-IV enzyme inhibition activity. At 10.0 μM concentration the percentage inhibition by these test compounds were observed around 17.3%, 28.4%, 24.1%, 20.4% and 19.1%, respectively whereas their IC_{50} values were calculated to be around 35.6, 21.3, 26.2, 32.7 and 29.1 μM, respectively.

In conclusion, we have designed and synthesized series of amine derivatives of 16-dehydropregnenolone and evaluated as inhibitors of DPP-IV. Compounds (**1**, **9**, **13**, **15** and **16**) were found to have significant inhibitory effect on DPP-IV enzyme.

Representative method for the preparation of amine derivative of acetylated 16-dehydropregnenolone: 16-Dehydropregnenolone acetate (100 mg, 0.28 mmol) and different amine (10 equiv) were taken in a round bottom flask (50 ml). The reaction mixture was stirred for 4 h at 45 °C. After cooling, diluted with water and extracted with EtOAc. Combined organic extract was washed with brine and dried over Na_2SO_4 and evaporated to furnish crude product, which purified by chromatography on silica gel using mixture of chloroform–methanol as eluting solvent to give the desired compounds **2–12**.

Compound **2**. This crude material was purified by flash chromatography (6: 94, MeOH/CHCl$_3$) to give pure compound **2** in 31% yield as yellow crystals, mp 168–170 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 5.29 (m, H-6), 4.53 (m, H-3), 4.12 (m, H-16), 3.12 (br, NH), 2.71 (m, H-17, 1′), 2.32 (m, H-4), 2.23 (s, H-21), 2.22 (m, CH$_3$CO), 2.04 (m, H-12), 1.97 (m, H-7), 1.84 (m, H-1, 2, 2′), 1.68 (m, H-15), 1.62 (m, H-11, 2), 1.57 (m, H-7), 1.47 (m, H-11), 1.46 (m, H-8), 1.43 (m, H-12), 1.23 (m, H-15), 1.17 (m, H-14), 1.10 (m, H-9), 1.08 (m, H-1), 0.96 (s, H-18), 0.96 (t, J = 7.0 Hz, H-3′), 0.57 (s, H-19); ^{13}C NMR:(75 MHz, CDCl$_3$) δ 207.4 (C-20), 142.6 (C-5), 121.86 (C-6), 72.4 (C-3), 68.9 (C-17), 58.4 (C-16), 55.6 (C-14), 51.6 (C-9), 49.9 (C-1′), 46.3 (C-13), 43.0 (C-4), 39.5 (C-12), 38.5 (C-1), 37.8 (C-10), 32.8 (C-8), 32.6 (C-7), 32.3 (C-2), 31.5 (C-21), 30.6 (C-15), 22.0 (C-2′), 21.0 (C-11), 19.9 (C-18), 14.4 (C-19), 11.4 (C-3′); MS: *m/z* 416 (M+H)$^+$.

Compound **3**. This crude material was purified by flash chromatography (3: 97, MeOH/CHCl$_3$) to give pure compound **3** in 42% yield as brown crystals, mp 174–176 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 5.35 (m, H-6), 4.17 (m, H-16), 3.37 (m, H-3), 2.85 (m, H-17, 1′), 2.27 (m, H-4), 2.23 (s, H-21), 2.04 (m, H-12), 1.97 (m, H-7), 1.84 (m, C-1, 2, 2′), 1.68 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.48 (m, H-2), 1.46 (m, H-8), 1.47 (m, H-11), 1.43 (m, H-12), 1.23 (m, H-15), 1.17 (m, H-14), 1.08 (m, H-1), 1.03 (s, H-18), 1.03 (t, J = 6.9 Hz, H-3′), 0.98 (s, H-9), 0.66 (s, H-19); ^{13}C NMR:(75 MHz, CDCl$_3$) δ 206.0 (C-20), 170.5 (OCOCH$_3$), 139.6 (C-5), 121.9 (C-6), 73.7 (C-3), 67.5 (C-17), 57.1 (C-16), 53.9 (C-14), 49.2 (C-9), 49.0 (C-1′), 45.1 (C-13), 38.3 (C-4), 38.0 (C-12), 36.8 (C-1), 36.5 (C-10), 31.4 (C-8), 31.4 (C-7), 31.3 (C-2), 29.8 (C-21), 27.6 (C-15), 21.6 (OCO*CH*$_3$), 20.7 (C-2′), 19.8 (C-11), 19.3 (C-18), 14.1 (C-19), 11.4 (C-3′); MS: *m/z* 374 (M+H)$^+$.

Compound **4**. This crude material was purified by flash chromatography (8: 92, MeOH/CHCl$_3$) to give pure compound **4** in 36% yield as white crystals, mp 159–160 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 7.24 (m, H-6′, 8′), 7.14 (m, H-5′, 7′, 9′), 5.30 (m, H-6), 4.55 (m, H-3), 4.01 (m, H-16), 2.92 (d, J = 6.1 Hz, H-17), 2.79 (m, H-1′), 2.68 (m, H-1′), 2.58 (t, J = 7.8 Hz, H-3′), 2.29 (m, H-4), 2.16 (m, H-7, 12) 2.08 (s, H-21), 2.00 (m, CH$_3$CO), 1.84 (m, H-1, 2, 2′), 1.67 (m, H-15), 1.63 (m, H-11, 2), 1.57 (m, H-7), 1.46 (m, H-8), 1.46 (m, H-11), 1.36 (m, H-12), 1.23 (m, H-15), 1.08 (m, H-1, 14), 0.95 (s, H-18), 0.90 (m, H-9), 0.53 (s, H-19). ^{13}C NMR: (75 MHz, CDCl$_3$) δ 205.9 (C-20), 139.7 (C-5), 139.1 (C-4′), 128.9 (C-6′, 8′), 128.1 (C-5′, 9′), 125.9 (C-7′), 122.2 (C-6), 73.6 (C-3), 67.9 (C-17), 57.7 (C-16), 54.4 (C-14), 49.6 (C-9), 47.2 (C-1′), 44.8(C-13), 38.5(C-4), 38.1 (C-12), 35.9 (C-1), 36.6 (C-10, 3′), 31.6 (C-8), 30.9 (C-7), 31.6 (C-2), 29.8 (C-21), 28.4 (C-2′), 27.7 (C-15), 20.8 (C-11), 19.8 (C-18), 14.3 (C-19); MS: *m/z* 492 (M+H)$^+$.

Compound **5**. This crude material was purified by flash chromatography (5: 95, MeOH/CHCl$_3$) to give pure compound **5** in 42% yield as yellow solid, mp 165–167 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 7.29 (m, H-6′, 8′), 7.20 (m, H-5′, 7′, 9′), 5.32 (m, H-6), 4.05 (m, H-16), 3.47 (m, H-3), 3.06 (d, J = 8.2 Hz, H-17), 2.80 (m, H-1′), 2.74 (m, H-1′), 2.69 (t, J = 7.8 Hz, H-3′), 2.28 (m, H-4), 2.20 (s, H-21), 2.16 (m, H-7, 12), 2.05 (m, H-1, 2′), 1.88 (m, H-2) 1.81 (m, H-15), 1.70 (m, H-11, 2), 1.67 (m, H-7), 1.46 (m, H-8), 1.44 (m, H-11), 1.36 (m, H-12), 1.26 (m, H-15), 1.13 (m, H-1, 14), 0.99 (s, H-18), 0.88 (m, H-9), 0.60 (s, H-19); ^{13}C NMR: (75 MHz, CDCl$_3$) δ 205.6 (C-20), 170.5 (OCOCH$_3$), 139.9 (C-5), 139.6 (C-4′), 128.8 (C-6′, 8′), 128.3 (C-5′, 9′), 126.5 (C-7′), 122.1 (C-6), 73.9 (C-3), 67.9 (C-17), 57.3 (C-16), 54.0 (C-14), 49.2 (C-9), 47.2 (C-1′), 44.9 (C-13), 38.2 (C-4), 38.0 (C-12), 36.8 (C-1), 36.5 (C-10, 3′), 31.3 (C-8), 31.3 (C-7), 31.3 (C-2), 29.7 (C-21), 28.2 (C-2′), 27.7 (C-15), 21.5 (OCO*CH*$_3$), 20.8 (C-11), 19.3 (C-18), 14.1 (C-19); MS: *m/z* 450 (M+H)$^+$.

Compound **6**. This crude material was purified by flash chromatography (5: 95, MeOH/CHCl$_3$) to give pure compound **6** in 60% yield as yellow needles mp 138–139 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 5.35 (m, H-6), 4.58 (m, H-3), 3.71 (t, J = 4.4 Hz, H-3′, 5′), 3.61 (m, H-16), 2.74 (d, J = 8.6 Hz, H-17), 2.53 (m, H-2′, 6′), 2.32 (m, H-4, 2′, 6′), 2.18 (s, H-21), 2.02 (m, CH$_3$CO), 2.02 (m, H-12), 1.97 (m, H-7), 1.84 (m, H-1, 2), 1.68 (m, H-15), 1.62 (m, H-11, 2), 1.57 (m, H-7), 1.50 (m, H-8), 1.50 (m, H-11), 1.50 (m, H-12), 1.18 (m, H-15), 1.13 (m, H-14), 1.04 (m, H-1), 1.00 (s, H-18), 0.86 (m, H-9), 0.64 (s, H-19); ^{13}C NMR: (75 MHz, CDCl$_3$) δ 208.1 (C-20), 170.7 (OCOCH$_3$), 139.8.1 (C-5), 122.3 (C-6), 73.7 (C-3), 67.8 (C-17), 65.9 (2X C-2′), 58.5 (C-16), 55.1 (2X C-1′), 54.1 (C-14), 51.6 (C-9), 44.9 (C-13), 39.1 (C-4), 38.2 (C-12), 37.0 (C-10), 36.7 (C-1), 31.9 (C-8), 31.8 (C-21), 31.7 (C-2, 7), 22.2 (C-15), 21.7 (OCO*CH*$_3$), 21.1 (C-11), 19.5 (C-18), 14.6 (C-19); MS: *m/z* 444 (M+H)$^+$.

Compound **7**. This crude material was purified by flash chromatography (8: 92, MeOH/CHCl$_3$) to give pure compound **7** in 75% yield as yellow crystals, mp 177–179 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 5.27 (m, H-6), 4.02 (m, H-16), 3.44 (m, H-3), 2.90 (d, J = 7.1 Hz, H-17), 2.68 (m, H-1′), 2.22 (m, H-4), 2.22 (s, H-21), 2.04 (m, H-12), 1.96 (m, H-7), 1.83 (m, H-1, 2, 2′), 1.69 (m, H-15), 1.64 (m, H-11), 1.55 (m, H-7), 1.47 (m, H-2), 1.47 (m, H-11), 1.46 (m, H-8), 1.44 (m, H-12), 1.25–1.18 (H-14, 15, 3′-11′) 1.08 (m, H-1), 0.95 (s, H-18), 0.84 (t, J = 6.9 Hz, H-12′), 0.98 (s, H-9), 0.58 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) δ 206.4 (C-20), 140.8 (C-5), 121.1 (C-6), 71.5 (C-3), 68.2 (C-17), 57.1 (C-16), 54.1 (C-14), 49.5 (C-9), 47.6 (C-1′), 45.1 (C-13), 42.1 (C-4), 38.5 (C-12), 37.2 (C-1), 36.7 (C-10), 32.0 (C-8), 32.0 (C-7), 31.6 (C-2), 31.5 (C-21), 30.0 (C-15), 29.8-29.3 (C-3′-10′) 26.5 (C-2′), 22.8 (C-11′), 21.0 (C-11), 19.5 (C-18), 14.2 (C-19), 14.2 (C-12′); MS: *m/z* 500 (M+H)$^+$.

Compound **8**. This crude material was purified by flash chromatography (12: 88, MeOH/CHCl$_3$) to give pure compound **8** in 75% yield as yellow sticky solid. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 5.30 (m, H-6), 3.92 (m, H-16), 3.46 (m, H-3), 2.90 (t, *J* = 6.0 Hz, H-1′), 2.75 (m, H-17), 2.50 (t, *J* = 6.0 Hz, H-2′) 2.42 (s, H-3′), 2.17 (m, H-4), 2.16 (s, H-21), 2.03 (m, CH$_3$CO), 1.94 (m, H-7), 1.90 (m, H-12), 1.85 (m, H-2), 1.82 (m, H-1), 1.68 (m, H-15), 1.62 (m, H-11, 2), 1.60 (m, H-15), 1.57 (m, H-7), 1.57 (m, H-12), 1.47 (m, H-11),1.46 (m, H-8), 1.14 (m, H-14), 1.02 (m, H-1), 1.00 (m, H-9), 0.96 (s, H-18), 0.58 (s, H-19). ^{13}C NMR: (75 MHz, CDCl$_3$) δ_C 206.5 (C-20), 142.6 (C-5), 122.5 (C-6), 72.7 (C-3), 69.2 (C-17), 58.1 (C-16), 55.6 (C-14), 53.8 (C-1′), 52.7 (C-3′), 51.0 (C-9), 46.4 (C-13), 43.4 (C-4), 39.8 (C-12), 37.7 (C-1), 37.5 (C-10), 36.3 (C-2′), 33.9 (C-7), 32.7 (C-8), 32.5 (C-2), 31.6 (C-21), 22.8 (C-15), 21.7 (C-11), 19.6 (C-18), 14.4 (C-19); MS: *m/z* 445 (M+H)$^+$.

Compound **9**. This crude material was purified by flash chromatography (6: 94, MeOH/CHCl$_3$) to give pure compound **9** in 78% yield as brown crystals, mp 156–158 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 7.34–7.21 (m), 5.35 (m, H-6), 3.85 (m, H-16), 3.71 (d, *J* = 12.2 Hz, H-1′), 3.59 (d, *J* = 12.2 Hz, H-1′), 3.51 (m, H-3), 2.5 (d, *J* = 6.5 Hz, H-17), 2.27 (m, H-4), 2.16 (s, H-21), 2.16 (m, H-12), 1.97 (m, H-7), 1.84 (m, H-1, 2), 1.68 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.48 (m, H-2), 1.46 (m, H-8), 1.46 (m, H-11), 1.43 (m, H-12), 1.24 (m, H-15), 1.16 (m, H-14), 1.08 (m, H-1), 1.01 (s, H-18), 0.98 (s, H-9), 0.66 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) δ 208.8 (C-20), 141.0 (C-5), 139.5 (C-2′), 128.7 (C-3′, 7′), 128.5 (C-4′, 6′), 127.4 (C-5′), 121.4 (C-6), 72.2 (C-3), 71.7 (C-17), 57.7 (C-16), 54.8 (C-14), 53.0 (C-1′), 50.1 (C-9), 45.1 (C-13), 42.4 (C-4), 39.0 (C-12), 37.4 (C-1), 36.7 (C-10), 32.8 (C-8), 32.0 (C-7), 31.7 (C-2), 31.7 (C-21), 31.7 (C-15), 21.0 (C-11), 19.6 (C-18), 14.6 (C-19); MS: *m/z* 422 (M+H)$^+$.

Compound **10**. This crude material was purified by flash chromatography (12: 88, MeOH/CHCl$_3$) to give pure compound **10** in 61% yield as yellow crystals, mp 138-140 °C. ^{1}H NMR: 300 MHz, CD$_3$OD) δ_H 5.33 (m, H-6), 4.00 (m, H-16), 3.57 (m, H-1′), 3.37 (m, H-3), 2.85 (t, *J* = 5.9 Hz, H-3′), 2.55 (d, *J* = 8.8 Hz, H-17), 2.27 (m, H-4), 2.23 (s, H-21), 2.05 (m, H-12), 1.96 (m, H-7), 1.85 (m, C-1, 2, 2′), 1.67 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.48 (m, C-2), 1.47 (m, H-11), 1.45 (m, C-8), 1.43 (m, H-12), 1.22 (m, H-15), 1.17 (m, H-14), 1.08 (m, C-1), 1.03 (s, H-18), 1.03 (t, *J* = 6.9 Hz, H-3′), 0.98 (s, H-9), 0.66 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) δ_C 206.5 (C-20), 142.4 (C-5), 122.1 (C-6), 73.8 (C-3), 67.9 (C-17), 57.4 (C-16), 55.8 (C-14), 51.2 (C-9), 54.1 (C-2′), 53.4 (C-1′), 45.8 (C-13), 45.1 (2X C-4′), 43.1 (C-4), 38.3 (C-12), 38.1 (C-10), 36.6 (C-1), 33.2 (C-7), 32.7 (C-8), 32.4 (C-2), 31.4 (C-21), 24.6 (C-15), 21.5 (C-11), 19.4 (C-18), 14.1 (C-19); MS: *m/z* 406 (M+H)$^+$.

Compound **11**. This crude material was purified by flash chromatography (8: 92, MeOH/CHCl$_3$) to give pure compound **11** in 67% yield as pale yellow crystals, mp 145–147 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 5.37 (m, H-6), 4.17 (m, H-16), 3.37 (m, H-3), 2.85 (m, H-17, 2′), 2.27 (m, H-4), 2.24 (s, H-21), 2.04 (m, H-12), 1.96 (m, H-7), 1.84 (m, H-1, 2, 3′), 1.68 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.48 (m, H-2), 1.47 (m, H-11), 1.46 (m, H-8), 1.43 (m, H-12), 1.31 (m, H-6′), 1.23 (m, H-15), 1.22 (m, H-4′, 5′) 1.17 (m, H-14), 1.08 (m, H-1), 1.02 (s, H-18), 1.00 (t, *J* = 6.8, H-7′), 0.99 (s, H-9), 0.67 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) δ_C 207.9 (C-20), 142.6 (C-5), 121.9 (C-6), 69.7 (C-3), 69.1 (C-17), 58.7 (C-16), 55.6 (C-14), 51.2 (C-9), 46.5 (C-13), 43.2 (C-4), 39.4 (C-12), 38.5 (C-1′), 37.8 (C-1), 37.6 (C-10), 33.7 (C-7), 32.9 (C-2), 32.4 (C-8), 31.5 (C-21), 30.6 (C-2′), 29.6 (C-3′), 29.4 (C-4′), 22.7 (C-15), 22.3 (C-7′), 21.9 (C-5′), 21.7 (C-11), 19.9 (C-18), 14.8(C-6′), 14.3 (C-19); MS: *m/z* 430 (M+H)$^+$.

Compound **12**. This crude material was purified by flash chromatography (15: 85, MeOH/CHCl$_3$) to give pure compound **12** in 68% yield as white powder, mp 158–160 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 5.30 (m, H-6), 4.08 (m, H-16), 3.36 (m, H-3), 3.20 (m, H-1′), 3.13 (t, *J* = 6.9 Hz, H-3′), 3.14 (m, H-5′) 2.92 (d, *J* = 7.0 Hz, H-17), 2.20 (m, H-4), 2.23 (s, H-21), 2.02 (m, H-12), 1.97 (m, H-7), 1.89 (m, H-2′), 1.85 (m, H-1, 2), 1.70 (m, H-15), 1.64 (m, H-11), 1.62 (m, H-7), 1.48 (m, H-2), 1.46 (m, H-8), 1.45 (m, H-11), 1.43 (m, H-12), 1.23 (t, *J* = 6.9 Hz, H-6′), 1.12 (m, H-15), 1.05 (m, H-14), 1.00 (m, H-1), 0.93 (s, H-18), 0.82 (s, H-9), 0.55 (s, H-19); ^{13}C NMR: (75 MHz, CDCl$_3$) δ_C 209.3 (C-20), 142.8 (C-5), 122.4 (C-6), 72.5 (C-3), 69.3 (C-17), 58.5 (C-16), 55.5 (C-14), 51.1 (C-9), 52.1 (C-3′), 48.6 (2 X C-5′), 46.3 (C-13), 43.0 (C-4), 39.5 (C-12), 37.8 (C-1), 37.6 (C-1′), 37.7 (C-10), 33.4 (C-7), 32.8 (C-8), 32.8 (C-21), 32.3 (C-2), 25.3 (C-2′), 22.9 (C-15), 21.9 (C-11), 19.9 (C-18), 14.5 (C-19), 9.6 (2 X C-6′); MS: *m/z* 445 (M+H)$^+$.

Representative method for synthesis of benzaldehyde derivative of 16-dehydropregnenolone (**13** and **14**): To a stirred solution of 16-dehydropregnenolone acetate (100 mg, 0.28 mmol) in 50% aqueous potassium hydroxide solution in ethanol (5 ml) was added the aldehyde (66.4 mg, 0.4 mmol). The reaction mixture was stirred at room temperature for 24 h. The resultant mixture was quenched with ice-cold water, acidified with 1 N HCl, extracted with ethyl acetate. The combined organic extract was washed with brine and dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by chromatography over silica gel using mixture of hexane–ethyl acetate as eluting solvent to give pure compounds **13**–**14**.

Compound **13**. This crude material was purified by flash chromatography (15:85 EtOAc/hexane) to give pure compound **13** in 76% yield as light yellow crystals, mp 182–184 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 7.91 (d, *J* = 16.5 Hz, H-22), 7.28 (d, *J* = 1.2 Hz, H-24), 7.22 (dd, *J* = 7.7, 1.2 Hz, H-28), 6.91 (d, *J* = 7.7 Hz, H-27), 6.67 (m, H-16), 6.38 (d, *J* = 16.5 Hz, H-21), 5.40 (m, H-6), 3.88 (s, –OCH$_3$), 3.88 (s, –OCH$_3$), 3.58 (m, H-3), 2.45 (m, H-4), 2.41 (m, H-12), 2.38 (m, H-4), 2.28 (m, H-15), 2.04 (m, H-15), 2.00 (m, H-7), 1.84 (m, H-1, 2), 1.62 (m, H-11), 1.61 (m, H-7), 1.50 (m, H-2), 1.46 (m, H-8), 1.47 (m, H-11), 1.35 (m, H-12), 1.17 (m, H-14), 1.08 (m, H-1), 1.05 (s, H-18), 1.01 (s, H-9), 0.87 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) 193.4 (C-20), 153.4 (C-17), 148.5 (C-26), 147.2 (C-25), 146.3 (C-22), 143.7 (C-16), 141.6 (C-5), 129.0 (C-23), 123.7 (C-28), 122.5 (C-21), 121.2 (C-6), 114.3 (C-27), 112.8 (C-24), 72.1 (C-3), 56.5 (C-14), 56.1 (OCH$_3$), 56.0 (OCH$_3$) 50.7 (C-9), 46.9 (C-13), 42.5 (C-4), 34.8 (C-12), 37.3 (C-1), 36.9 (C-10), 32.8 (C-8), 32.6 (C-7), 31.8 (C-15), 32.1 (C-2), 22.9 (C-11), 19.5 (C-18), 14.3 (C-19); MS: *m*/*z* 463 [M+H]$^+$.

Compound **14**. This crude material was purified by flash chromatography (12:88 EtOAc/Hexane) to give pure compound **14** in 81% yield as yellow needles, mp 146–147 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 7.91 (d, *J* = 16.5 Hz, H-22), 7.62 (d, *J* = 7.9 Hz, H-25, 27), 7.27 (d, *J* = 7.9 Hz, H-24, 28), 6.38 (d, *J* = 16.5 Hz, H-21), 5.34 (m, H-6), 4.19 (m, H-16), 3.90 (m, H-1′), 3.37 (m, H-3), 3.36 (m, H-2″), 2.90 (m, H-17), 2.28 (m, H-4), 2.06 (m, H-12), 1.97 (m, H-7), 1.83 (m, H-1, 2), 1.67 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.50 (m, H-2), 1.46 (m, H-8), 1.47 (m, H-11), 1.43 (m, H-12), 1.27 (d, *J* = 7.0 Hz, H-1″, 3″), 1.23 (m, H-15), 1.17 (m, H-14), 1.10 (t, *J* = 6.8 Hz, H-2′), 1.08 (m, H-1), 1.03 (s, H-18), 0.98 (*s*, H-9), 0.68 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) 194.1 (C-20), 153.2 (C-17), 147.2 (C-22), 146.8 (C-26), 143.3 (C-16), 142.1 (C-5), 129.5 (C-23), 129.2 (C-25, 27), 123.7 (C-24, 28), 121.9 (C-21), 121.2 (C-6), 71.9 (C-3), 56.4 (C-14), 50.7 (C-9), 46.4 (C-13), 42.7 (C-4), 34.6 (C-12), 37.4 (C-1), 36.7 (C-10), 33.4 (C-29), 32.6 (C-8), 32.3 (C-7), 32.1 (C-2), 31.7 (C-15), 23.9 (C-30, 31), 22.7 (C-11), 19.5 (C-18), 14.3 (C-19); MS: *m*/*z* 491 [M+H]$^+$.

Representative method for the synthesis of Aza-annulation on the 16-dehydropregnenolone: The mixture of 16-dehydropregnenolone acetate (100 mg, 0.28 mmol) in *N*-propylamine (10 equiv) was heated for 4 h at 45 °C. The unreacted *N*-propylamine was evaporated under reduced pressure, the residue obtained was dissolved in ethanol and 50% w/v aqueous potassium hydroxide solution and *p*-methoxy benzaldehyde (0.05 mL 0.42 mmol) was added in reaction mixture. The reaction mixture was stirred at room temperature for 24 h. The resultant mixture was diluted with ice-cold water and acidified with 1 N HCl, extracted with ethyl acetate. The combined organic extract was washed with brine, dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by flash chromatography using mixture of chloroform–methanol (98: 02) as eluting solvent to afford pure compound **15**. By the similar procedure compounds **16** and **17** were prepared from the corresponding aromatic aldehydes.

Compound **15**. This crude material was purified by flash chromatography (8: 92, MeOH/CHCl$_3$) to give pure compound **15** in 71% yield as white crystals, mp 221–223 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 7.31 (d, J = 7.9 Hz, H-24, 28), 6.79 (d, J = 7.9 Hz, H-25, 27), 5.36 (m, H-6), 4.50 (m, H-22), 4.17 (m, H-16), 3.87 (s, OCH$_3$), 3.51 (m, H-3), 2.85 (m, H-17, 1′), 2.27 (m, H-4), 2.05 (m, H-21), 2.04 (m, H-12), 1.97 (m, H-7), 1.84 (m, H-1, 2, 2′), 1.68 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.48 (m, H-2), 1.47 (m, H-11), 1.46 (m, H-8), 1.43 (m, H-12), 1.25 (t, J = 6.9 Hz, H-3′), 1.23 (m, H-15), 1.17 (m, H-14), 1.08 (m, H-1), 1.03 (s, H-18), 1.01 (s, H-19), 0.98 (s, H-9); ^{13}C NMR: (75 MHz, CD$_3$OD) δ 208.5 (C-20), 159.6 (C-26), 142.3 (C-5), 131.6 (C-23), 129.7 (C-24, 28), 121.7 (C-6), 113.6 (C-25, 27), 79.9 (C-22), 71.7 (C-3), 30.8 (C-21), 65.3 (C-17), 56.7 (OCH$_3$), 56.4 (C-14), 56.3 (C-16), 50.8 (C-9), 50.2 (C-1′), 44.8 (C-13), 42.8 (C-4), 39.3 (C-12), 37.2 (C-1), 34.8 (C-10), 32.4 (C-8), 31.9 (C-7), 31.3 (C-2), 26.1 (C-15), 20.7 (C-2′), 20.5 (C-11), 19.4 (C-18), 15.1 (C-19), 14.6 (C-3′); MS: m/z 492 [M+H]$^+$.

Compound **16**. This crude material was purified by flash chromatography (5: 95, MeOH/CHCl$_3$) to give pure compound **16** in 61% yield as white crystals, mp 232–234 °C. ^{1}H NMR: (300 MHz, CD$_3$OD) δ_H 7.50 (d, J = 7.9 Hz, H-25, 27), 7.27 (d, J = 7.9 Hz, H-24, 28), 3.36 (m, H-2″), 5.35 (m, H-6), 4.60 (m, H-22), 4.17 (m, H-16), 3.37 (m, H-3), 2.85 (m, H-17, 1′), 2.27 (m, H-4), 2.04 (m, H-21), 2.04 (m, H-12), 1.97 (m, H-7), 1.84 (m, H-1, 2, 2′), 1.68 (m, H-15), 1.62 (m, H-11), 1.57 (m, H-7), 1.48 (m, H-2), 1.46 (m, H-8), 1.47 (m, H-11), 1.43 (m, H-12), 1.27 (d, J = 7.0 Hz, H-1″, 3″), 1.23 (m, H-15), 1.17 (m, H-14), 1.08 (m, H-1), 1.03 (s, H-18), 1.03 (t, J = 6.9 Hz, H-3′), 0.98 (s, H-9), 0.66 (s, H-19); ^{13}C NMR: (75 MHz, CD$_3$OD) δ 208.2 (C-20), 146.4 (C-26), 141.4 (C-5), 132.9 (C-23), 128.7 (C-24, 28), 127.2 (C-25, 27), 121.6 (C-6), 80.6 (C-22), 71.9 (C-3), 65.5 (C-17), 56.6 (C-16), 56.4 (C-14), 50.7 (C-9), 50.1 (C-1′), 44.7 (C-13), 42.7 (C-4), 39.1 (C-12), 37.1 (C-1), 34.7 (C-10), 33.6 (C-29), 32.3 (C-8), 31.2 (C-7), 31.8 (C-2), 30.4 (C-21), 23.8 (C-30, 31), 26.4 (C-15), 20.7 (C-2′), 20.4 (C-11), 19.4 (C-18), 15.2 (C-19), 14.5 (C-3′); MS: m/z 504 [M+H]$^+$.

Compound **17**. This crude material was purified by flash chromatography (10: 90, MeOH/CHCl$_3$) to give pure compound **17** in 75% yield as white crystals mp 226–228 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H 6.68 (d, J = 3.16 Hz, H-25), 6.68 (d, J = 3.16 Hz, H-24), 5.31 (m, H-6), 4.40 (m, H-22), 3.49 (m, H-3), 3.40 (m, H-16), 2.52 (d, J = 6.9 Hz, H-17), 2.45 (m, H-1′), 2.27 (m, H-4), 2.04 (m, H-21), 2.04 (m, H-12), 1.97 (m, H-7), 1.85 (m, H-1, 2, 2′), 1.69 (m, H-15), 1.63 (m, H-11), 1.56 (m, H-7), 1.47 (m, H-2), 1.46 (m, H-8), 1.46 (m, H-11), 1.43 (m, H-12), 1.23 (m, H-15), 1.17 (m, H-14), 1.08 (m, H-1), 1.03 (s, H-18), 1.13 (t, J = 6.9 Hz, H-3′), 1.00 (s, H-9), 0.59 (s, H-19); ^{13}C NMR: (75 MHz, CDCl$_3$) δ 208.7 (C-20), 154.4 (C-26), 144.8 (C-23), 141.6 (C-5), 121.2 (C-25), 121.0 (C-24, 6), 79.7 (C-22), 71.8 (C-3), 67.5 (C-17), 65.0 (C-16), 56.5 (C-14), 50.6 (C-9), 50.0 (C-1′), 44.5 (C-13), 42.2 (C-4), 38.8 (C-12), 37.2 (C-1), 34.7 (C-10), 32.4 (C-8), 31.8 (C-7), 31.6 (C-2), 30.3 (C-21), 26.1 (C-15), 20.8 (C-2′), 19.5 (C-11), 19.4 (C-18), 15.5 (C-19), 14.5 (C-3′); MS: m/z 486 [M+H]$^+$.

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