



(43) International Publication Date
12 November 2015 (12.11.2015)

(10) International Publication Number
WO 2015/170336 A1

- (51) **International Patent Classification:**
C07D 233/54 (2006.01) *A61P 35/00* (2006.01)
- (21) **International Application Number:**
PCT/IN2015/000188
- (22) **International Filing Date:**
29 April 2015 (29.04.2015)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
1249/DEL/2014 9 May 2014 (09.05.2014) IN
- (71) **Applicant:** COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH [—/IN]; Anusandhan Bhawan, Rafi Marg, New Delhi 110001 (IN).
- (72) **Inventors:** GOGOI, Sanjib; Csir-, Neist, Jorhat 785001 (IN). BORUAH, Romesh Chandra; Csir-, Neist, Jorhat 785001 (IN). SAIKIA, Pallabi; Csir-, Neist, Jorhat 785001 (IN). ADDLAGATTA, Anthony; Csir-, Iict, Hyderabad 500007 (IN). SADDANAPU, Venkateshwarlu; Csir-, Iict, Hyderabad 500007 (IN).
- (74) **Agents:** RAE, Konpal et al.; Lakshmikumaran & Sridharan, B6/10, Safdarjung Enclave, New Delhi 110029 (IN).

- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

(54) **Title:** 16 α -HETEROARYL PREGNENOLONE ACETATE AND A PROCESS FOR PREPARATION THEREOF

(57) **Abstract:** The present invention relates to microwave assisted facile Michael addition of benzimidazole and imidazole to steroidal α,β -unsaturated ketones under solvent-free condition to afford corresponding Michael adducts 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1) and 16 α -(1H-imidazol-1-yl)-pregnenolone acetate (2). Compound 1 showed in vitro cytotoxic activities almost comparable to the drug doxorubicin against cervical HeLa cancer cell line (compound 1, IC₅₀ = 8.3317, doxorubicin IC₅₀ = 7.8126), prostate DU 205 cancer cell line (compound 1, IC₅₀ = 12.0192, doxorubicin IC₅₀ = 9.1194) and breast cancer MCF-7 cell line (compound 1, IC₅₀ = 8.2855, doxorubicin IC₅₀ = 7.5094).



WO 2015/170336 A1

16 α -HETEROARYL PREGNENOLONE ACETATE AND A PROCESS FOR PREPARATION THEREOF

FIELD OF INVENTION

5 [0001] The present invention relates to 16 α -heteroaryl-1-yl pregnenolone acetate and a process for its preparation. The present invention particularly relates to compounds of 16 α -(1*H*-benzimidazol-1-yl)-pregnenolone acetate (1) and 16 α -(1*H*-imidazol-1-yl)-pregnenolone acetate (2). The present invention also relates to a process for the preparation of said compounds from 16-dehydropregnenolone acetate (16-DPA). More
10 particularly the present invention relates to the anticancerous activity of the said compounds (1 and 2).

BACKGROUND OF THE INVENTION

[0002] Benzimidazole, imidazole and their derivatives are very important pharmacophores in medicinal chemistry showing a wide range of biological activities
15 like antibacterial, antifungal, anticancer, antiviral, antituberculosis and antiinflammatory and antiageing agents.

[0003] Synthesis of several modified steroids comprising aza heterocyclic rings either fused or linked to the steroid framework have received considerable attention in the recent years due to their wide range of biological activities. These steroidal
20 heterocycles are well known as inhibitors of enzymes such as 5 α -reductase, 17 α -lyase and aromatase. Steroids comprising heterocyclic rings such as pyridine, imidazole, benzimidazole and diazine ring linked to 17-position show anticancer activity. Even 16-substituted steroids either by heterocycles or nonheterocyclic compounds have shown wide range of pharmacological activities. For example many 16*E*-
25 arylidenosteroids are reported which show *in vitro* activity against many types of tumor cells. Even, 16-substituted steroids with imidazole heterocycle such as 16 β -imidazolyl androsterone derivatives are known which are synthesized using 17-oxo-5-

androst-3 β -ol as the starting material and these compounds are found to exhibit moderate cytotoxic effect in sixty cancer cell lines derived from nine cancer types.

[0004] Conjugated addition of benzimidazole/imidazole to α,β -unsaturated carbonyl compounds are known using Lewis acid catalyzed addition, base catalyzed addition, high pressure promoted addition, iodine catalyzed addition, and acid catalyzed addition reactions. Unfortunately, many of these processes suffer from limitations, such as the use of expensive catalyst, expensive reagents, harsh conditions, relatively long reaction times, high catalyst loading, tedious work-up procedures and use of hazardous organic solvents. Hence, the development of a sustainable environment as an alternative procedure for Michael addition reaction avoiding the use of harmful solvents and expensive catalysts remains a challenging task to organic chemists. On the other hand, microwave-assisted organic synthesis (MAOS) which is recognized as a "green" technology has been applied as a very efficient way to accelerate the course of many organic reactions, producing high yields, higher selectivity, and lower quantities of side products.

[0005] Reference may be made to *G A Potter, S E Barrie, M Jarman & M G Rowlands J. Med. Chem. 1995, 38, 2463* wherein, steroidal compounds having 17-(3-pyridyl) substituent together with 16,17-double bond were synthesized which showed potent inhibition of human testicular 17 α -hydroxylase-C_{17,20}-lyase.

[0006] Another reference may be made to *V D Handratta, T S Vasaitis, V C O Njar, L K Gediya, R Kataria, P Chopra, D Newman, Jr. R Farquhar, Z Guo, Y Qiu & A M H Brodie J. Med. Chem. 2005, 48, 2972* wherein, the syntheses and biological activities of novel 17-benzoxazoles and 17-diazines were reported. The 17-benzimidazole steroidal compounds were shown to exhibit potent in vitro activities for CYP17 inhibition, AR antagonism and inhibition of prostate cancer cell growth.

[0007] Still another reference may be made to *R D Bruno, T S Vasaitis, L K Gediya, P Purushottamachar, A M Godbole, Z Ates-Alagoz, A M H Brodie & V C O Njar*

Steroids 2011, 76 1268 wherein it was found that 17-benzimidazole steroidal compound VN/124-1 was a potent inhibitor of human prostate cancer tumor growth and was remarkably more effective than castration or compound abiraterone that is currently undergoing phase III clinical trials in prostate cancer patients.

- 5 [0008] Still another reference may be made to *R Bansal & S Guleria Steroids* 2008, 73, 1391 wherein the synthesis of 16-substituted steroidal compounds such as 16E-[3-methoxy-4-(2-aminoethoxy)-benzylidene]androstene derivatives and their cytotoxicity studies were reported.

- 10 [0009] Still another reference may be made to *R Bansal, S Guleria, S Thota, S L Bodhankar, M R Patwardhan, C Zimmer, R W Hartmann & A L Harvey Steroids* 2012, 77, 621 wherein, syntheses of 16 β -imidazolyl-17-keto steroids and its 16 β -imidazolyl-17 β -hydroxyl derivatives were reported which exhibited moderate cytotoxic effects in sixty cancer cell lines derived from nine cancers types.

- 15 [0010] Still another reference may be made to *J P Morrison, C A Stein, D S Casebier, J Morrison, C Stein and D Casebier WO/2011/017534*, wherein, compounds, pharmaceutical compositions and medicaments comprising such compounds, and methods of using such compounds that decrease androgen biosynthesis, decrease androgen receptor signaling and decrease androgen receptor sensitivity are reported.

- 20 [0011] Still another reference may be made to *R Bansal, S Guleria, G Narang and H R Wolfgang WO/2007/031833*, wherein a novel series of imidazolyl substituted steroidal and indan-1-one derivatives are reported.

[0012] Still another reference may be made to *A Brodie and V C O Njar US5994335*, wherein 17-azolyl steroids are reported to be useful as androgen synthesis inhibitors.

OBJECTIVES OF THE INVENTION

- 25 [0013] The main object of the present invention is to provide 16 α -heteroaryl-1-yl pregnenolone acetate and a process for preparation of the compounds.

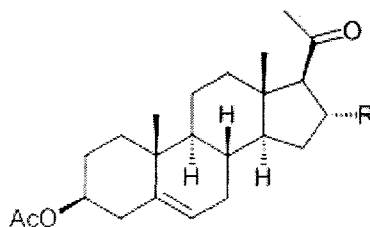
[0014] Another object of the present invention is to provide compounds 16 α -(1*H*-benzimidazol-1-yl)-pregnenolone acetate (1) and 16 α -(1*H*-imidazol-1-yl)-pregnenolone acetate (2).

[0015] Another object of the present invention is to provide a process for the preparation of 16 α -(1*H*-benzimidazol-1-yl)-pregnenolone acetate (1) and 16 α -(1*H*-imidazol-1-yl)-pregnenolone acetate (2) from 16-dehydropregnenolone acetate (16-DPA) without using solvent and catalyst under microwave irradiation.

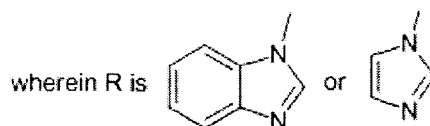
[0016] Still another objective of the present invention is to evaluate the *in vitro* cytotoxic activities of compound 1 and 2 against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line in comparison to the drug doxorubicin.

SUMMARY OF INVENTION

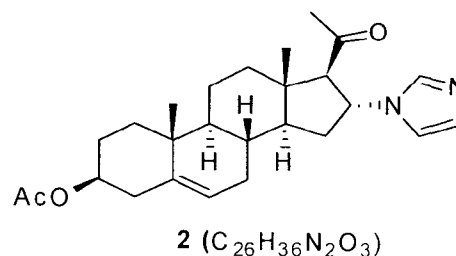
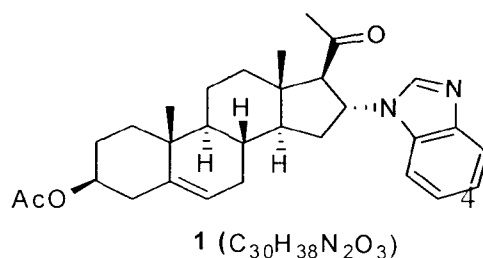
[0017] Accordingly the present invention provides a compound of formula A,



15 Formula A



[0018] In an embodiment of the present invention compound, wherein structural formula of representative compound comprising are:



20

[0019] In another embodiment of the present invention a compound wherein the formula is represented by the group of the following compounds:-

- a) 16 α -(1*H*-benzimidazol-1-yl)-pregnenolone acetate (1)
- b) 16 α -(1*H*-imidazol-1-yl)-pregnenolone acetate (2)

5

[0020] In another embodiment of the present invention a compound wherein 16 α -(1*H*-benzimidazol-1-yl)-pregnenolone acetate (1) and 16 α -(1*H*-imidazol-1-yl)-pregnenolone acetate (2) exhibited *in vitro* cytotoxic activity against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line.

10 [0021] In another embodiment of the present invention a compound wherein cytotoxic activities of compound 1 with IC₅₀= 8.3317 against HeLa cancer cell line, IC₅₀= 12.0192 against prostate DU 205 cancer cell line and IC₅₀= 8.2855 against breast cancer MCF-7 cell line.

15 [0022] In yet another embodiment of the present invention a compound wherein cytotoxic activities of compound 2 with IC₅₀= 48.2584 against HeLa cancer cell line, IC₅₀= 37.8069 against prostate DU 205 cancer cell line and IC₅₀= 20.0288 against breast cancer MCF-7 cell line.

20 [0023] In yet another embodiment of the present invention a process for the preparation of compound of formula (1) wherein the said process comprises of following steps:-

- a) mixing α,β -unsaturated ketone 16-DPA with compounds selected from benzimidazole or imidazole in mortar,
- b) irradiating the mixture obtained in step a) in a microwave reactor at a frequency of 2450 MHz for a time period ranging between 10 to 30 minutes,
- c) purifying the crude product obtained in step b) by silica gel column chromatography using EtOAc-hexane taken in the ratio of 1:4.

25

[0024] In another embodiment of the present invention a process wherein the compounds are prepared by solvent free and catalyst-free method by the Michael addition of benzimidazole to 16-DPA under microwave irradiation.

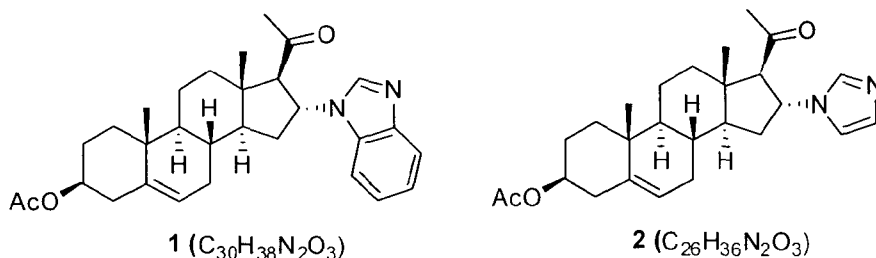
[0025] In another embodiment of the present invention a process, wherein the yield of the compound of formula A is as high as 47% to 86%.

BRIEF DESCRIPTION OF DRAWINGS & FIGURES

[0026] Figure 1 shows the synthesis of compounds 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (**1**) and 16 α -(1H-imidazol-1-yl)-pregnenolone acetate (**2**) from 16-DPA.

[0027] Figure 2 shows the key NOESY correlations and relative stereochemistry of compounds **1** and **2**. From this correlation the *trans*-orientation of the substituents in **16** and **17** positions was confirmed.

DETAILED DESCRIPTION OF THE INVENTION



[0028] The invented compounds 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (**1**) and 16 α -(1H-imidazol-1-yl)-pregnenolone acetate (**2**) are of pregnenolone acetate. One benzimidazole group and imidazole group are substituted at 16-position in compounds **1** and **2**, respectively. These compounds were characterized by ^1H NMR, ^{13}C NMR and mass spectroscopy. These substituents at 16 position are α -oriented which are *trans*-orientated with respect to the acetyl group at C-17 position. This *trans*-orientation is proved by the NOESY spectra of both the compounds **1** and **2**. Compound **1** is a yellow solid, (m.p.: 118 $^{\circ}\text{C}$) and compound **2** is a brown solid (m.p.:

131 °C). Both the compounds **1** and **2** show *in vitro* cytotoxic activity against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line.

- [0029] Accordingly, the present invention provides a process for the preparation of 16 α -(1*H*-benzimidazol-1-yl)-pregnenolone acetate (**1**) and 16 α -(1*H*-imidazol-1-yl)-pregnenolone acetate (**2**) by the solvent-free and catalyst-free Michael addition of nitrogen containing heterocycles benzimidazole and imidazole to 16-dehydropregnenolone acetate under microwave irradiation. The present invention also provides biological evaluation of compounds **1** and **2** as cytotoxic agent.
- [0030] The reaction of equimolar amount of 16-DPA (**1**) and benzimidazole in protic solvents ethanol and isopropanol under thermal heating conditions could not provide the product **1** (Figure 1). Similar results were obtained using nonprotic solvents acetonitrile and dimethylformamide under reflux condition (8 hours). Interestingly, when the reaction was performed in a solvent-free condition, under thermal heating (140 °C) for 8 hours, the product **1** was obtained in satisfactory yield (40%). It was noteworthy to observe that finely ground equimolar amounts of **1** and benzimidazole under microwave irradiation (720 Watt) for 10 minutes afforded **1** in 69% yield. The formation of 16 α -substituted *N*-heterocycles having trans orientation with respect to the acetyl group at C-17 position of the steroid moiety was determined by comparing the optical rotational data of these hetero steroids with the reported 16-substituted steroidal heterocycles by Gould *et al.* (*D Gould, E L Shapiro, L E Finckenor, F Gruen & E B Hershberg J. Am. Chem. Soc. 1956, 78, 3158*). The α orientation of benzimidazole at 16-position and the β orientation of acetyl group at 17-position was then confirmed by NOESY spectrum of compound **1**. In the NOESY spectrum of compound **1**, the presence of correlation of CH₃-18 protons with H-16 and CH₃-18 protons with H-21 protons and the absence of correlation between CH₃-18 protons with H-17 also indicated that H-16 and H-17 were β and α oriented respectively in compound **1** (Figure 2). Similarly, the reaction of 16-DPA with imidazole under

microwave irradiation (720 Watt) for 10 minutes afforded Michael adduct **2** in 86% yield under microwave irradiation (Figure 1).

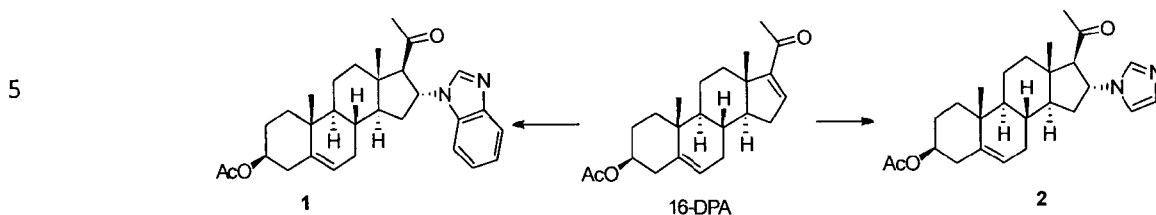


Figure 1

10 **[0031]** In vitro cytotoxic activity studies of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (**1**):

[0032] These cell lines were procured from ATCC in 2009. Catalog numbers HeLa (ATCC®CCL-2™)-15th passage cells used for the present experiment. DU 145 (ATCC® HTB-81™)-12 the passage cells used for the present experiment. MCF7
15 (ATCC® HTB-22™)-15 the passage cells used for the present experiment.

[0033] Cellular viability in the presence of test compounds was determined by MTT-micro cultured tetrazolium assay. The cells seeded to flat bottom 96 (1000cells/100 μ l) well plates and cultured in the medium containing 10% serum and allowed to attach and recover for 24 hours in a humid chamber containing 5% CO₂. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; sigma catalog No M2128)
20 was dissolved in PBS at 5 mg/mL, filtered to sterilize and remove a small amount of insoluble residue present in MTT. Then different concentrations of the compound were added to the cells. After 48 hours, stock MTT solution (10 μ l) was added to the culture plate. Cells were again kept in CO₂ incubator for 2 hours. After incubation, 100 μ l of
25 DMSO was added and mixed. The absorbance was read at 562 nm in a plate reader. The results were represented as percentage of cytotoxicity/viability. All the experiments were carried out in triplicates. From the percentage of cytotoxicity the IC-

50 value was calculated (*S Myadaraboina, M Alla, V Saddanapu, V R Boenaa & A Addlagatta. Eur. J. Med. Chem. 2010, 45, 5208*).

[0034] As showed in Table 1, compound **1** displayed good cytotoxicity to the tested cancer cell lines. Compound **1** had IC₅₀ values of 8.3317, 12.0192 and 8.2855 μ M against HeLa cell line, DU 145 cell line and MCF-7 cancer cell lines, respectively which is almost comparable to the drug doxorubicin. Compound **2** exhibited moderate activity against the tested cell lines. It had IC₅₀ values of 48.2584, 37.8069 and 20.0288 μ M against HeLa cell line, DU 145 cell line and MCF-7 cancer cell lines, respectively.

Compound ID	IC ₅₀ in μ M HeLa cell line at 10 μ M conc.	IC ₅₀ in μ M DU 145 cell line at 10 μ M conc.	IC ₅₀ in μ M MCF-7 cell line at 10 μ M conc.
1	8.3317	12.0192	8.2855
2	48.2584	37.8069	20.0288
Doxorubicin	7.8126	9.1194	7.5094

10 **Table 1.** IC₅₀ values of compound **1 & 2** determined by MTT assay

[0035] The steroidal conjugated enone 16-DPA could be converted into corresponding Michael adduct **1** and **2** with benzimidazole and imidazole, respectively in high yield under microwave irradiation. The steroidal ketone **1** has shown cytotoxic activity similar to drug doxorubicin against HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line.

EXAMPLES

[0036] The following examples are given by illustrations and should not construe the scope of the invention.

Example-I

20 [0037] Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (**1**):

α,β -Unsaturated ketone 16-DPA (356 mg, 1.0 mmol) and benzimidazole (118 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. (Yield 69%, 327 mg). The crude product can be purified by neutral alumina also using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adducts **1**. (Yield 67%, 318 mg). Yellow solid, m.p.: 117 °C.;

R_f = 0.3 (EtOAc/Hexane = 1:4); $[\alpha]_D$ = -6.6 (c, 0.5, CHCl₃); IR (CHCl₃, cm⁻¹): 2927, 2856, 1732, 1709, 1655, 1361, 1250, 1033, 762; ¹H NMR (CDCl₃, 300 MHz): δ 8.10 (s, 1H), 7.79 (dd, 1H, J = 6.8 & 3.5 Hz), 7.50 (dd, 1H, J = 8.2 & 2.0 Hz), 7.33-7.23 (m, 2H), 5.59-5.53 (m, 1H), 5.41-5.38 (m, 1H), 4.66-4.60 (m, 1H), 3.06 (d, 1H, J = 8.5 Hz), 2.09 (s, 3H), 2.05 (s, 3H), 1.07 (s, 3H), 2.36-0.89 (m, 17H), 0.81 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz): δ 206.2, 170.6, 144.0, 142.1, 139.9, 132.7, 122.9, 122.2, 121.7, 120.5, 110.5, 73.7, 70.6, 58.4, 56.6, 54.6, 49.7, 45.2, 38.6, 38.0, 36.9, 36.6, 32.0, 31.6, 27.7, 21.4, 20.7, 19.3, 18.4, 13.9; MS (EI, m/z) = 474 (M⁺), 414 (M⁺-60). Anal. calcd. for C₃₀H₃₈N₂O₃: C, 75.92; H, 8.07; N, 5.90. Found: C, 76.22; H, 8.18; N, 6.03.

[0038] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

16 DPA (356 mg, 1.0 mmol) and imidazole (68 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **2**. Brown solid, m.p.: 130 °C. Yield 86% (365 mg); R_f : 0.4 (EtOAc/Hexane = 3:7); $[\alpha]_D$ = -8.4 (c 0.5, CHCl₃); IR (CHCl₃) cm⁻¹: ν 2943, 2854, 1731, 1705, 1649, 1364, 1247, 1034, 754; ¹H NMR (CDCl₃, 300 MHz): δ 7.55 (s, 1H), 7.03 (s, 1H), 6.91 (s, 1H), 5.40-5.37 (m, 1H), 5.30-5.22 (m, 1H), 4.67-4.57 (m, 1H), 2.80 (d, 1H, J = 7.8 Hz), 2.10 (s, 3H), 2.04 (s, 3H), 1.04 (s, 3H), 0.73 (s, 3H), 2.40-0.89 (m, 17H); ¹³C NMR (CDCl₃, 75 MHz): δ 206.0, 170.6, 141.4, 139.8 (2C), 121.7 (2C),

73.7, 72.8, 56.1, 55.8, 49.5, 45.2, 38.0, 36.6, 33.6, 31.9, 31.5, 29.7, 29.4, 27.7, 22.7, 21.4, 20.6, 19.3, 14.1; MS (EI) m/z = 364 (M^+ -60), 296 [$(M^+$ -60)-imidazole]. Anal. calcd. for $C_{26}H_{36}N_2O_3$: C, 73.55; H, 8.55; N, 6.60; Found: C, 73.69; H, 8.51; N, 6.80.

Example-II

5 **[0039]** Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1):

α,β -Unsaturated ketone 16-DPA (2.136 g, 6.0 mmol) and benzimidazole (708 mg, 6.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. Yellow solid, m.p.: 119 °C. Yield 68% (1.93 g).

[0040] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

15 16 DPA (2.136 g, 6.0 mmol) and imidazole (408 mg, 6.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **2**. Brown solid, m.p.: 131 °C. Yield 85% (2.16 g).

Example-III

20 **[0041]** Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1):

α,β -Unsaturated ketone 16-DPA (4.272 g, 12.0 mmol) and benzimidazole (1.42 g, 12.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. Yellow solid, m.p.: 118 °C. Yield 69% (3.92 g).

[0042] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

16 DPA (4.272 g, 12.0 mmol) and imidazole (816 mg, 12.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford
5 steroidal Michael adduct **2**. Brown solid, m.p.: 130 °C. Yield 83% (4.22 g).

Example-IV

[0043] Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1):

α,β -Unsaturated ketone 16-DPA (356 mg, 1.0 mmol) and benzimidazole (118 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (540
10 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. (Yield 47%, 223 mg). Yellow solid, m.p.: 117 °C.

[0044] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

15

16 DPA (356 mg, 1.0 mmol) and imidazole (68 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (540 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal
20 Michael adduct **2**. Brown solid, m.p.: 130 °C. Yield 62% (263 mg).

Example-V

[0045] Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1):

α,β -Unsaturated ketone 16-DPA (356 mg, 1.0 mmol) and benzimidazole (118 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (540
25 Watt, 2450 MHz) for 20 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. (Yield 52%, 247 mg). Yellow solid, m.p.: 117 °C.

[0046] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

16 DPA (356 mg, 1.0 mmol) and imidazole (68 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (540 Watt, 2450 MHz) for 20 minutes.

- 5 On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **2**. Brown solid, m.p.: 130 °C. Yield 66% (280 mg).

Example-VI

[0047] Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1):

- 10 α,β -Unsaturated ketone 16-DPA (356 mg, 1.0 mmol) and benzimidazole (118 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt, 2450 MHz) for 20 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. (Yield 69%, 327 mg). Yellow solid,
15 m.p.: 116 °C.

[0048] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

16 DPA (356 mg, 1.0 mmol) and imidazole (68 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (720 Watt, 2450 MHz) for 20 minutes.

- 20 On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **2**. Brown solid, m.p.: 131 °C. Yield 86% (365 mg).

Example-VII

[0049] Preparation of 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (1):

- 25 α,β -Unsaturated ketone 16-DPA (356 mg, 1.0 mmol) and benzimidazole (118 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (900 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude

product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **1**. (Yield 65%, 308 mg). Yellow solid, m.p.: 116 °C.

[0050] Preparation of 3 β -acetoxy-16 α -(1H-imidazol-1-yl)-17 β -acetyl-androst-5-ene (2):

16 DPA (356 mg, 1.0 mmol) and imidazole (68 mg, 1.0 mmol) were mixed intimately in a mortar and irradiated in a microwave reactor (900 Watt, 2450 MHz) for 10 minutes. On completion of the reaction (by TLC), the crude product was purified by silica gel column chromatography using EtOAc-hexane (1:4) as the eluent to afford steroidal Michael adduct **2**. Brown solid, m.p.: 132 °C. Yield: 79% (335 mg).

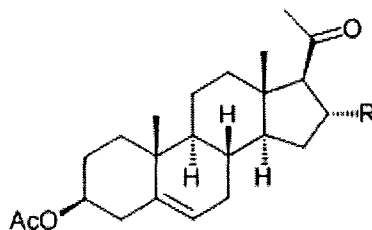
ADVANTAGES OF THE PRESENT INVENTION

[0051] The main advantages of the present invention are:

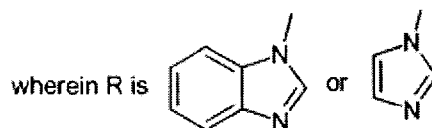
1. The compound 16 α -(1H-benzimidazol-1-yl)-pregnenolone acetate (**1**) exhibited cytotoxic activity against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line similar to drug doxorubicin.
2. The compound 16 α -(1H-imidazol-1-yl)-pregnenolone acetate (**2**) exhibited cytotoxic activity against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line.
3. Incorporation of a benzimidazole group and imidazole group at 16-position of steroidal D-ring via solvent and catalyst free Michael addition reaction of benzimidazole and imidazole with commercially available 16-DPA molecule under microwave irradiation.
4. Incorporation of a benzimidazole/imidazole group at 16-position affords 16 α -substituted heterosteroid which has *trans*-orientation with respect to 17-acetyl group.

We claim:

1. A compound of formula A,

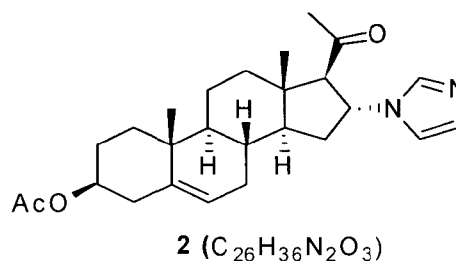
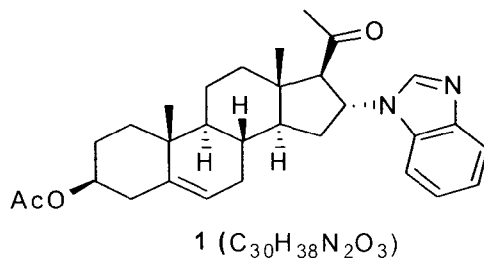


Formula A



5

2. A compound as claimed in claim 1, wherein structural formula of representative compounds comprising are:



10

3. A compound as claimed in claim 1 wherein the formula is represented by the group of the following compounds:-

a) 16α-(1H-benzimidazol-1-yl)-pregnenolone acetate (1)

b) 16α-(1H-imidazol-1-yl)-pregnenolone acetate (2)

4. A compound as claimed in claim 1 wherein 16α-(1H-benzimidazol-1-yl)-pregnenolone acetate (1) and 16α-(1H-imidazol-1-yl)-pregnenolone acetate (2) exhibited *in vitro* cytotoxic activity against cervical HeLa cancer cell line, prostate DU 205 cancer cell line and breast cancer MCF-7 cell line.

20

5. A compound as claimed in claim 1 wherein cytotoxic activities of compound 1 with IC_{50} = 8.3317 against HeLa cancer cell line, IC_{50} = 12.0192 against prostate DU 205 cancer cell line and IC_{50} = 8.2855 against breast cancer MCF-7 cell line.
6. A compound as claimed in claim 1 wherein cytotoxic activities of compound 2 with IC_{50} = 48.2584 against HeLa cancer cell line, IC_{50} = 37.8069 against prostate DU 205 cancer cell line and IC_{50} = 20.0288 against breast cancer MCF-7 cell line.
7. A process for the preparation of compound of formula (1) wherein the said process comprises of following steps:-
- a) mixing α,β -unsaturated ketone 16-DPA with compounds selected from benzimidazole or imidazole in mortar,
- b) irradiating the mixture obtained in step a) in a microwave reactor at a frequency of 2450 MHz for a time period ranging between 10 to 30 minutes,
- c) purifying the crude product obtained in step b) by silica gel column chromatography using EtOAc-hexane taken in the ratio of 1:4.
8. A process as claimed in claim 7 wherein the compounds are prepared by solvent free and catalyst-free method by the Michael addition of benzimidazole to 16-DPA under microwave irradiation.
9. A process as claimed in claim 7, wherein the yield of compound of formula A is as high as 47% to 86%.

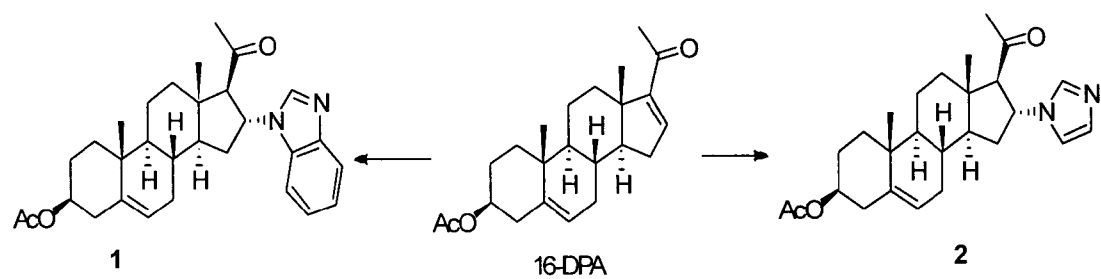


Figure 1

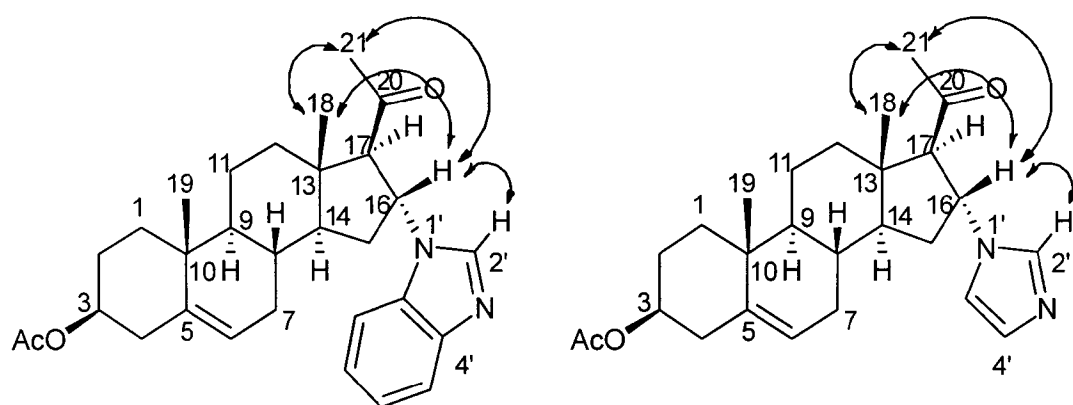


Figure 2. Key NOESY correlations and relative stereochemistry of compound **1** and **2**

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2015/000188A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D233/54 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RANJU BANSAL ET AL: "Design, synthesis and evaluation of novel 16-imidazolyl substituted steroidal derivatives possessing potent diversified pharmacological properties", STEROIDS, ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY, US, vol. 77, no. 6, 6 February 2012 (2012-02-06), pages 621-629, XP028410196, ISSN: 0039-128X, DOI: 10.1016/J.STEROIDS.2012.02.005 [retrieved on 2012-02-15] compound 9 ----- -/--	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 July 2015

Date of mailing of the international search report

30/07/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Bareyt, Sébastien

INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2015/000188

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/031833 A2 (COUNCIL SCIENT IND RES [IN]; BANSAL RANJU [IN]; GULERIA SHEETAL [IN];) 22 March 2007 (2007-03-22) cited in the application compound 29 -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IN2015/000188

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007031833 A2	22-03-2007	CN 101291950 A	22-10-2008
		EP 1934241 A2	25-06-2008
		JP 5238504 B2	17-07-2013
		JP 2009508839 A	05-03-2009
		US 2009137541 A1	28-05-2009
		WO 2007031833 A2	22-03-2007
