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MINI-REVIEW

AN UPDATE ON BIOTRANSFORMATIONAL STUDIES OF DYDROGESTERONE

Azizuddin

Department of Chemistry, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal Campus, Karachi-75300, Pakistan. E.mail: azizpobox1@yahoo.com

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ABSTRACT

Due to the result of enzymatic or metabolic activities by a living organism, the series of chemical reactions occur in a compound, especially a drug. Dydrogesterone (**1**) is a potent orally active progestogen. Biotransformation of dydrogesterone (**1**) by using human volunteers, rat, dog, mouse, rabbit and rhesus monkey, by fermentation with cell suspension cultures of *Sepedeonium ampullos* and *Azadirachta indica*, and fermentation with fungal cultures including *Fusarium solani*, *Cephalosporium aphidicola*, *Fusarium lini*, *Rhizopus stolonifer*, *Cunninghamella elegans* and *Gibberella fujikuroi* afforded metabolites **2**-**16**. This review article will provide detail about metabolites **2**-**16**, obtained by biotransformation of dydrogesterone (**1**) and have been reported up to 2012.

Keywords: *Azadirachta indica, Biotransformation, Cephalosporium aphidicola, Cunninghamella elegans, Dydrogesterone, Fusarium lini, Fusarium solani, Gibberella fujikuroi, Rhizopus stolonifer, Sepedeonium ampullos*

INTRODUCTION

Microorganisms employed such enzyme catalyzed reactions, which are well organized in metabolic pathways for the degradation or synthesis of a variety of chemical compounds. Such reactions are essential for maintaining the life functions of the cell, growth and reproduction. Nutrients are degraded in catabolic pathways yielding energy and small molecules as building blocks for anabolic metabolism. The energy provided by exothermic degradation steps is needed for the maintenance of viabi1ity and to support endothermic anabolic metabolism in which all the constituents needed for cell growth. Biotransformations occur in several organs of the body of living organisms including the kidneys, liver, skin, lungs, placenta and intestines. Substances absorbed in the gastrointestinal tract after oral administration must pass through the liver, where they can be transformed and thus eliminated before being distributed to other parts of the body. Now biotransformation has become an established method in organic chemical synthesis.

Dydrogesterone (**1**) (9β,10α-pregna-4,6-diene-3,20-dione) is a synthetic progestogen with potent oral activity that has been used for over 40 years in clinical practice. Dydrogesterone (**1**) is closely resembled to endogenous progesterone, which produces a complete secretory endometrium in an estrogen-primed uterus acting directly on the uterus. It helps to regulate the normal shedding of the uterus lining and healthy growth. Therefore, it may be useful in the treatment of irregular or painful menstrual cycle, menstrual disorders, endometriosis, premenstrual syndrome and infertility. Dydrogesterone (**1**) may also be used in hormone replacement therapy (HRT) to minimize the overgrowth of the womb lining. Therefore, it is also helpful to prevent miscarriage in women. Furthermore, dydrogesterone (**1**) is non-anabolic, non-androgenic, non-corticoid, non-estrogenic and is not excreted as pregnanediol.

BIOTRANSFORMED PRODUCTS OF DYDROGESTERONE (1)

Several articles have been published on biotransformation of dydrogesterone (**1**), and until 2012 fifteen metabolites **2**-**16** have been reported (Fig. 1). These metabolites **2**-**16** are also mentioned in Table 1. In this review article, an attempt has been made to establish a comparison among biotransformed products **2**-**16**.

Biotransformation of dydrogesterone (1) in human: Houki in 1966 have identified a urinary metabolite of dydrogesterone (**1**) in ovariectomized women by the injection of 6-dehydroretroprogesterone. He isolated a reduced product, which was identified as 20α-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**).

Van Leusden and Huberthus in 1970, obtainned two metabolites of dydrogesterone (**1**) in human after incubation of **1** with human placenta for 2 hours. The isolated substances, were identified as 20α-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**) and 17α-hydroxy-9β,10α-pregna-4,6-diene -3,20-dione (**3**).

Van Amsterdam and co-workers in 1980 investigated the urinary metabolites after oral administration of dydrogesterone (**1**) in healthy women of childbearing age. Among 43 isolates, three were positively identified as metabolites; 20α-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**), 16α-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione

(**4**) and 21-hydroxy-9β,10α-pregna-4,6-diene-3, 20-dione (**5**).

Biotransformation of dydrogesterone (1) in dog, rabbit, mouse, rhesus monkey and rat: Hiroshi and co-workers in 1968 identified urinary and biliary metabolite of dydrogesterone (**1**) in rabbit by the administration of 6-dehydroretroprogesterone, and isolated 20α-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**) as a metabolite.

De Bree, *et al*., in 1983a investigated the metabolic pattern of orally administrated radioactive dydrogesterone (**1**) in rhesus monkey, rabbit, dog, mouse and rat. Metabolites were extracted from the urine of rhesus monkey, rabbit, dog, mouse and rat, and also from bile of dog and rat, and separated. They identified 20α-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**), 17α-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**3**), 16α-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**4**) and 21-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**5**) as transformed products of **1**. The urinary patterns of mouse, dog and rat differed substantially, from each other as well as from those of monkey and rabbit. The patterns show certain similarities for rhesus monkey and rabbit to each other and to the human urinary pattern. All animals used were females.

De Bree, *et al*., in 1983b further isolated a unique metabolite of dydrogesterone (**1**) from the urine of rhesus monkey by intramuscular injection of dydrogesterone (**1**), was identified as 21-hydroxy-9β,10α-pregna-5,7-diene-3-ol-20-one (**6**). The rhesus monkey is the only specie to produce a metabolite **6** of dydrogesterone (**1**) not having retained the 4,6-diene-3-one configuration of the parent molecule. This metabolite was not found in the urine of the other animals or men.

Biotransformation of dydrogesterone (1) by cell suspension cultures of *Sepedeonium ampullos*: Mc Gregor and co-workers in 1972 isolated a biotransformed metabolite of **1** by using resting cell suspensions of *Sepedeonium ampullos*, which was identified as 16α-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**4**), a hydroxylated product.

Biotransformation of dydrogesterone (1) by cell suspension cultures of *Azadirachta indica*: Azizuddin and co-workers in 2008 subjected dydrogesterone (**1**) to biotransformation by incubation with the cell suspension cultures of *Azadirachta indica.* As a result of the reduction of C-20 ketonic group, a metabolite was identified as 20*R*-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**). This cell suspension culture was used for the first time for structural modification of dydrogesterone (**1**).

Biotransformation of dydrogesterone (1) by *Fusarium solani*: Neumann in 1965 reported incubation of dydrogesterone (**1**) with *Fusarium solani*, two metabolites were obtained as 17β-hydroxy-9β,10α-androsta-4,6-diene-3-one (**7**) and 9β,10α-androsta-4,6-diene-3,17-dione (**8**).

Biotransformation of dydrogesterone (1) by *Cephalosporium aphidicola*: Choudhary and co-workers in 2008 investigated metabolic pattern of the dydrogesterone (**1**) by using fungal strain of *Cephalosporium aphidicola*. Incubation of dydrogesterone (**1**) with *Cephalosporium aphidicola*, yielded five known metabolites; 20*R*-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**), 17β-hydroxy-9β,10α-androsta-4,6-diene-3-one (**7**), 9β,10α-angrosta-4,6-diene-3,17-dione (**8**), 17β-hydroxy-9β,10α-angrosta-1,4,6-triene-3-one (**9**) and 9β,10α -pregna-1,4,6-triene-3,20-dione (**10**). This was the first report for the synthesis of **2**, **7-10** by microbial transformation of dydrogesterone (**1**) using *C. aphidicola*. Metabolites **7** and **9** were found to be more potent against respiratory burst in human neutrophils than substrate **1**. Dydrogesterone (**1**) was found to be potent α-glucosidase inhibitor whereas its metabolite **9** was found to be moderately active against this enzyme (Azizuddin, *et al*., 2012).

Biotransformation of dydrogesterone (1) by *Rhizopus stolonifer*: Choudhary and co-workers in 2008 investigated metabolic pattern of dydrogesterone (**1**) by using of fungal strain *Rhizopus stolonifer*. Incubation of **1** with *Rhizopus stolonifer* yielded two metabolites; 9β-hydroxy-9β, 10α-pregna-4,6-diene-3,20-dione (**11**) and 8β-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**12**) by hydroxylation and 6α,7α-epoxy-8β-hydroxy-9β,10α-pregn-4-ene-3,20-dione (**13**) by hydroxylation with epoxidation. Metabolites **11** and **12** were found to be inactive against α-glucosidase enzyme than dydrogesterone (**1**) (Azizuddin, *et al*., 2012).

Azizuddin and co-workers in 2011 also reported a new dihydroxylated metabolite of 9β, 12β-dihydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**14**) with the same fungal strain.

Biotransformation of dydrogesterone (1) by *Fusarium lini*: Choudhary and co-workers in 2008 also investigated metabolic pathway of dydrogesterone (**1**) by using of fungal strain *Fusarium lini*. Fermentation of **1** with *Fusarium lini* yielded two metabolites 8β-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**12**) and 11β-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**15)** by mono-hydroxylation, and a new metabolite 11β, 15α-dihydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**16**) by di-hydroxylation. Metabolite **12** was

found to have more potent respiratory burst inhibition activity than substrate **1** in a human neutrophil-based cellular assay. Besides, metabolites **15** was found to be inactive against α-glucosidase enzyme than dydrogesterone (**1**) (Azizuddin, *et al*., 2012).

Biotransformation of dydrogesterone (1) by *Cunninghamella elegans*: Choudhary and co-workers in 2008 also reported the fermentation of dydrogesterone (**1**) by using the fungal strain *Cunninghamella elegans*. As a result, metabolite 9β-hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**11**) was obtained by hydroxylation of substrate **1**.

Biotransformation of dydrogesterone (1) by *Gibberella fujikuroi*: Azizuddin and Choudhary in 2012 investigated metabolic changes in dydrogesterone (**1**) by using fungal strain *Gibbe-rella fujikuroi*. Three metabolites; 20*R*-hydroxy-9β, 10α -pregna-4,6-diene-3-one (**2**), 17β-hydroxy-9β,10α-androsta-4,6-diene-3-one (**7**) and 9β,10α-angrosta-4,6-diene-3,17-dione (**8**) were obtained.

CONCLUSION

It is observed that dydrogesterone (**1**) retain its 4,6-diene-3-one structure in combination with 9β,10α-configuration during biotransformation, which is metabolically stable. Dydrogesterone (**1**) does not give 17α-hydroxylation, which explains its androgenic effects. Furthermore, aromatization does not occur in dydrogesterone (**1**), which is consistent due to absence of its estrogenic effects. This review is assumed that it will assist in comparative studies among biotransformed metabolites **2-16** of dydrogesterone (**1**), obtained from various ways of biotransformation.

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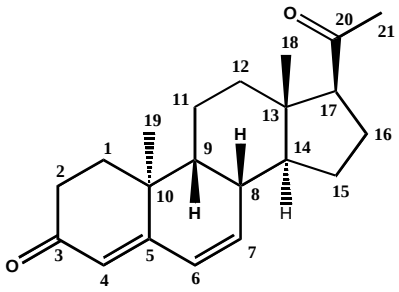
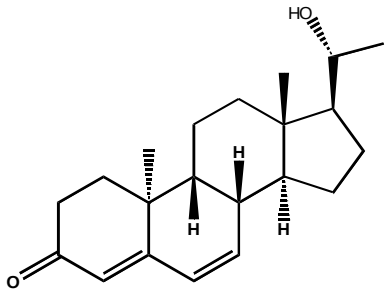


Dydrogesterone (1)



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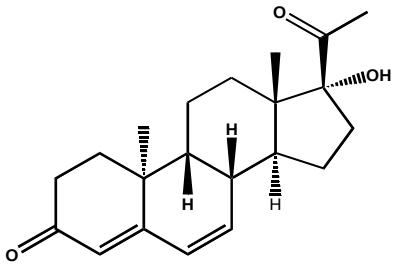

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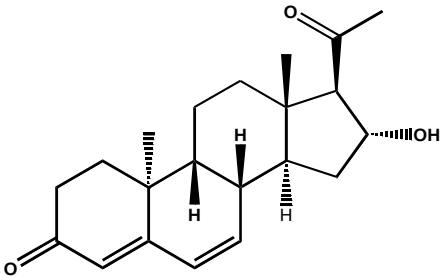

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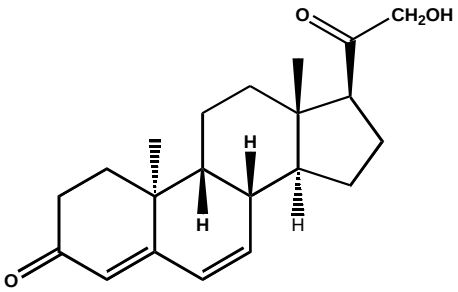

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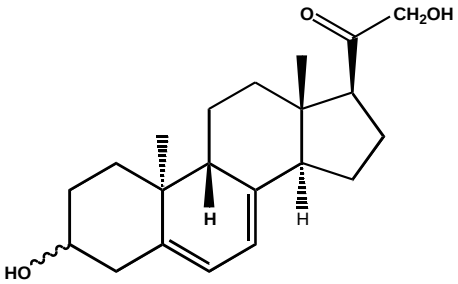

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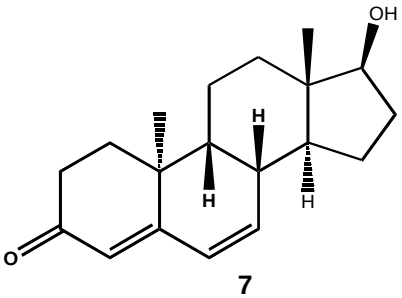

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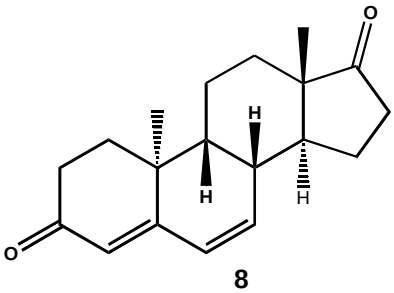

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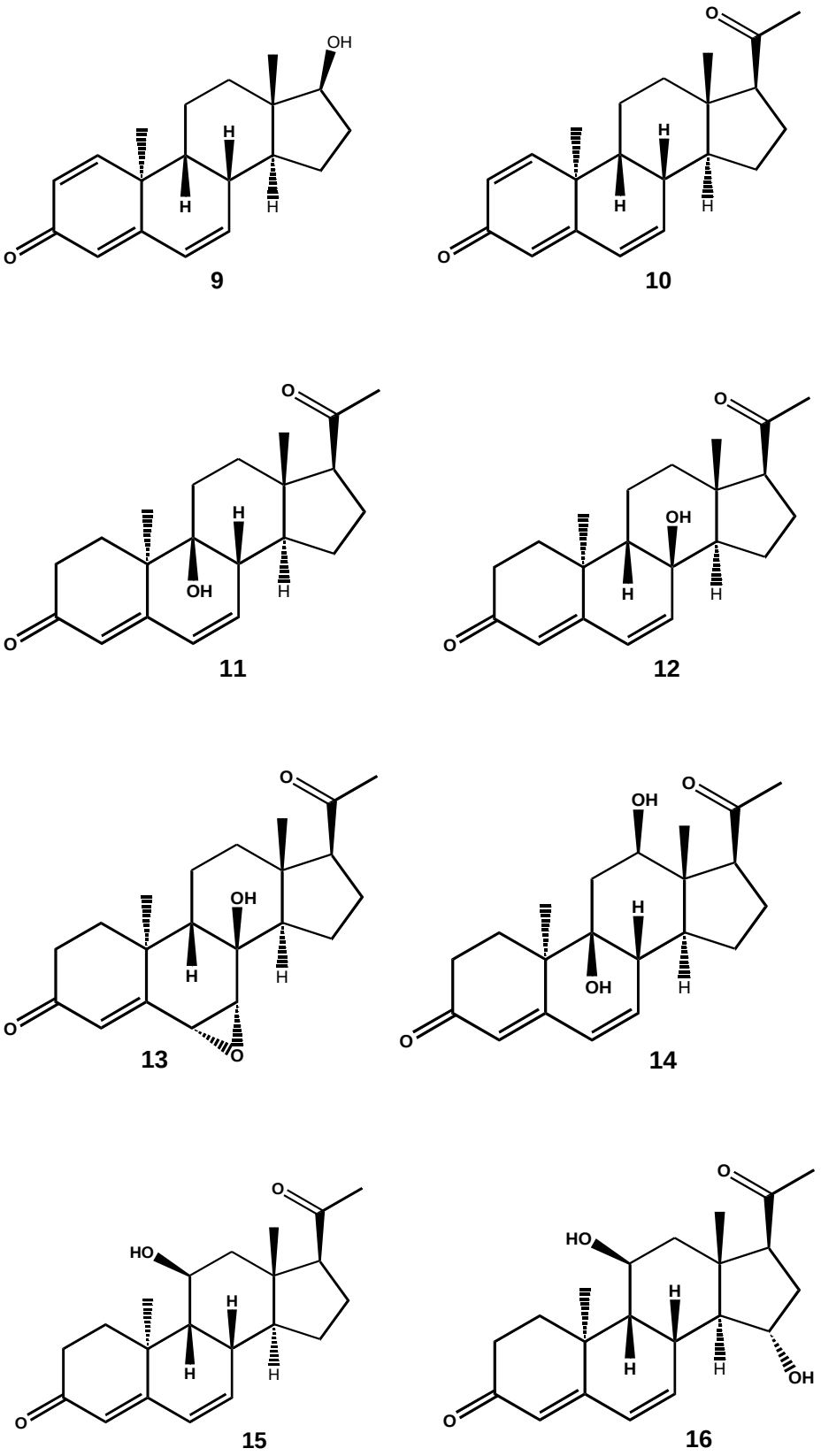

Fig. -1: Dydrogesterone (**1**) and its biotransformed metabolites **2-16**.

Table 1. Biotransformed metabolites, **2**-**16** of dydrogesterone (**1**).

S. No.	Biotransformed metabolites	Biotransformation pathways	References
1	20α-Hydroxy-9β,10α-pregna-4,6-diene -3-one (**2**)	From the urine of human; urine and bile of rabbit; incubation with human placenta; urine and bile of rat and dog; urine of mouse, rabbit and rhesus monkey; cell suspension cultures of *Azadiracta indica; Cephalosporium aphidicola* and *Gibberella fujikuroi*	De Bree, *et al*., 1983a; Azizuddin, *et al*., 2008; Azizuddin and Choudhary, 2012
2	17α-Hydroxy-9β,10α-pregna-4,6-diene-3, 20-dione (**3**)	From incubation with human placenta; urine and bile of rat and dog; urine of mouse, rabbit and rhesus monkey	De Bree, *et al*., 1983a
3	16α-Hydroxy-9β,10α-pregna-4,6-diene-3, 20-dione (**4**)	From the urine of human; cell suspension cultures of *Sepedonium ampullosporum*; urine and bile of rat and dog; urine of mouse, rabbit and rhesus monkey	Van Amsterdam, *et al*., 1980; De Bree, *et al*., 1983a
4	21-Hydroxy-9β,10α-pregna-4,6-diene-3, 20-dione (**5)**	From the urine of human; urine and bile of rat and dog; urine of mouse, rabbit and rhesus monkey	Van Amsterdam, *et al*., 1980; De Bree, *et al*., 1983a
5	21-Hydroxy-9β,10α-pregna-5, 7-diene-3-ol-20-one (**6**)	From the urine of rhesus monkey	De Bree, *et al*., 1983b
6	17β-Hydroxy-9β,10α-androsta-4,6-diene-3-one (**7**)	From the fungal strains of *Fusarium solani, Cephalosporium aphidicola* and *Gibberella fujikuroi*	Choudhary, *et al*., 2008; Azizuddin and Choudhary, 2012
7	9β,10α-Androsta-4,6-diene-3,17-dione (**8**)	From the fungal strains of *Fusarium solan*i, *Cephalosporium aphidicola* and *Gibberella fujikuroi*	Choudhary, *et al*., 2008; Azizuddin and Choudhary, 2012
8	17β-Hydroxy-9β,10α-androsta-1,4,6-triene-3-one (**9**)	From the fungal strain of *Cephalosporium aphidicola*	Choudhary, *et al*., 2008; Azizuddin, *et al*., 2012
9	9β,10α-Pregna-1,4,6-triene-3,20-dione (**10**)	From the fungal strain of *Cephalosporium aphidicola*	Choudhary, *et al*., 2008
10	9β-Hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**11**)	From the fungal strains of *Rhiozopus stolonifer* and *Cunninghamella elegan*	Choudhary, *et al*., 2008; Azizuddin, *et al*., 2012
11	8β-Hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**12**)	From the fungal strains of *Rhizopus stolonifer* and *Fusarium lini*	Choudhary, *et al*., 2008; Azizuddin, *et al*., 2012
12	6α,7α-Epoxy-8β-hydroxy-9β,10α-pregn-4-ene-3,20-dione (**13**)	From the fungal strain of *Rhizopus stolonifer*	Choudhary, *et al*., 2008
13	9*β*,12*β*-dihydroxy-9*β*,10*α*-pregna-4,6-diene-3,20-dione (**14**)	From the fungal strain of *Rhizopus stolonifer*	Azizuddin, *et al*., 2011
14	11β-Hydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**15**)	From the fungal strain of *Fusarium lini*	Choudhary, *et al*., 2008; Azizuddin, *et al*., 2012
15	11β,15α-Dihdyroxy-9β,10α-pregna-4,6-diene-3,20-dione (**16**)	From the fungal strain *of Fusarium lini*	Choudhary, *et al*., 2008