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Synthesis, Characterization and Pharmacological Evaluation of Ester Derivate of Prednisolone

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ABSTRACT

In the present study some novel ester derivates of prednisolone were synthesized as prodrugs and evaluated for their anti- inflammatory activity. This compound was prepared by reacting Ethyl-N-tert-butyloxycarbonylglycinate-protected amino acids. These novel prodrugs were also characterized with the help of usual analytical and spectral techniques (I.R spectroscopy & N.M.R. spectroscopy). These newly synthesized ester derivates of prodrugs were screened for their spectral techniques. All the above compounds showed significant anti- inflammatory effect at 50 mg/kg p.o. and the experimental data are statistically significant at $p< 0.05$ level. The pharmacological activities exhibited by synthesized novel compounds have confirmed that they may serve the purpose of being accepted as the novel therapeutic agents as anti-inflammatory agents.

Keywords: Prednisolone; Ester Derivates; Spectral Techniques; Anti- Inflammatory

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INTRODUCTION

In recent years, prednisolone analogues and derivatives have attracted strong attention due to their biological and pharmacological properties. Amino acid derived prednisolone derivatives possess a wide spectrum of biological applications such as antitumor, antimicrobial and anti-inflammatory etc. Prodrug is such a compound that after administration is metabolized into a pharmacologically active drug [1,2]. They are bioreversible derivatives of drug molecules that undergo an enzymatic and /or chemical transformation in *vivo* to release the active parent drug, which can then exert the desired pharmacological effect [2]. The development of prodrug is now well established as a strategy to improve the physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically potent compounds and thereby increase the develop ability and usefulness of a potential drug [3-5]. Instead of administrating a drug directly, a prodrug might be used instead to improve how a medicine is absorbed, distributed, metabolized and excreted [6,7]. Prodrugs are often designed to improve bioavailability when a drug itself is poorly absorbed from the gastrointestinal track [8] . In zoos, one third of all approved small molecular weight drugs were prodrugs [9] .The basic aim of prodrug design is to mark undesirable drug properties, such as low solubility in water or lipid membranes, low target selectivity, chemical instability, undesirable test irritation or pain after local administration presystemic metabolism and toxicity [10-12].

Amino acid derived esters derivates are the most common prodrugs and are activated by enzymatic hydrolysis[13] .Ester prodrug like prednisolone is most often used to enhance the lipophilicity, and thus the passive membrane permeability, of water soluble drugs by masking charged groups such as carboxylic acids, hydroxyl, thiol and phosphates [14, 15]. Once in the body, the ester bond is readily hydrolyzed by ubiquitous esterase's found in the blood, liver and other organs and tissues [16], including carboxylesterases, acetylcholinesterases, butyrylcholinesterases, paraoxonases and arylesterases.

Keeping in view the above facts, some novel ester derivates of prednisolone derived from various amino acids are synthesized characterized and their pharmacological activities are evaluated in the present study. The structures of five compounds were confirmed by elemental and spectral analysis.

MATERIALS AND METHOD

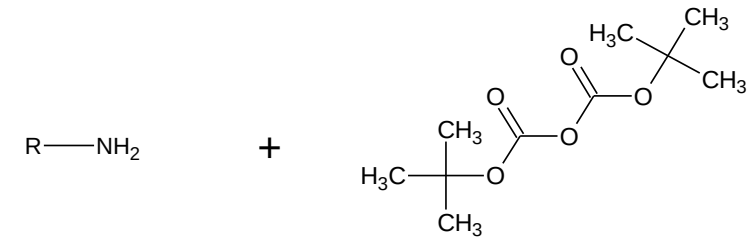
Experimental

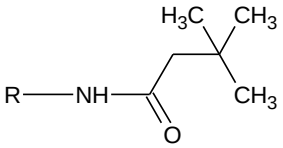
Melting points were determined by TEMPO apparatus in open capillary tubes. The purity of these prednisolone derived prodrugs was established by thin layer chromatography. H^1-NMR spectra

were recorded on Bruckner AC-300 MHz instrument using Me4Si (TMS) as internal standard (Chemical shift in **S**. ppm). The I.R spectra were recorded on perkin Elms 882 model. Such spectra were obtained with KBr pellets (**y** max in cm^{-1}). Aluminum TLC plates (E. Merk) were used and spots were visualized by exposure to UV radiation (260nm) and iodine vapors. Anhydrous Na_2SO_4 was used as drying agent.

Synthesis of Ethyl-N-tert-butyloxycarbonylglycinate (2)

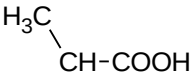
To a 2.0 L, three necked, round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, and two stoppers, charged 170 ml of saturated aq. $NaHCO_3$ solution, 34.2 g (0.585mol) of NaCl, 350 ml of chloroform, 6.3g (.084 mol) of glycine and stirred for 10 minute at RT. Charged 36.5 g (0.167mol) of BOC anhydride at RT and again stirred for 15 min. Heated the reaction mixture to reflux and maintained u/reflux for 20 hrs under. Cooled the reaction mass to RT and transferred the reaction mass to a separator funnel and separated the layers. Collected the organic layer and extracted Aq. Layer with chloroform (2 ×100 ml). Combined the organic layers and washed with (2× 100 ml) water. Dried the organic layer over Na_2SO_4, filtered and concentrated U/Vacc. At 40-50 ^oC to afford a total of 12.2g (0.698 mol) of oil as BOC-protected glycine (2)

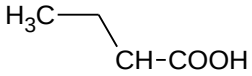


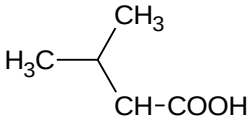


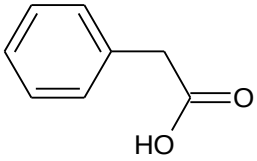
BOC Protected aminoacid

R= H_2C—COOH

R= 

R= 

R= 

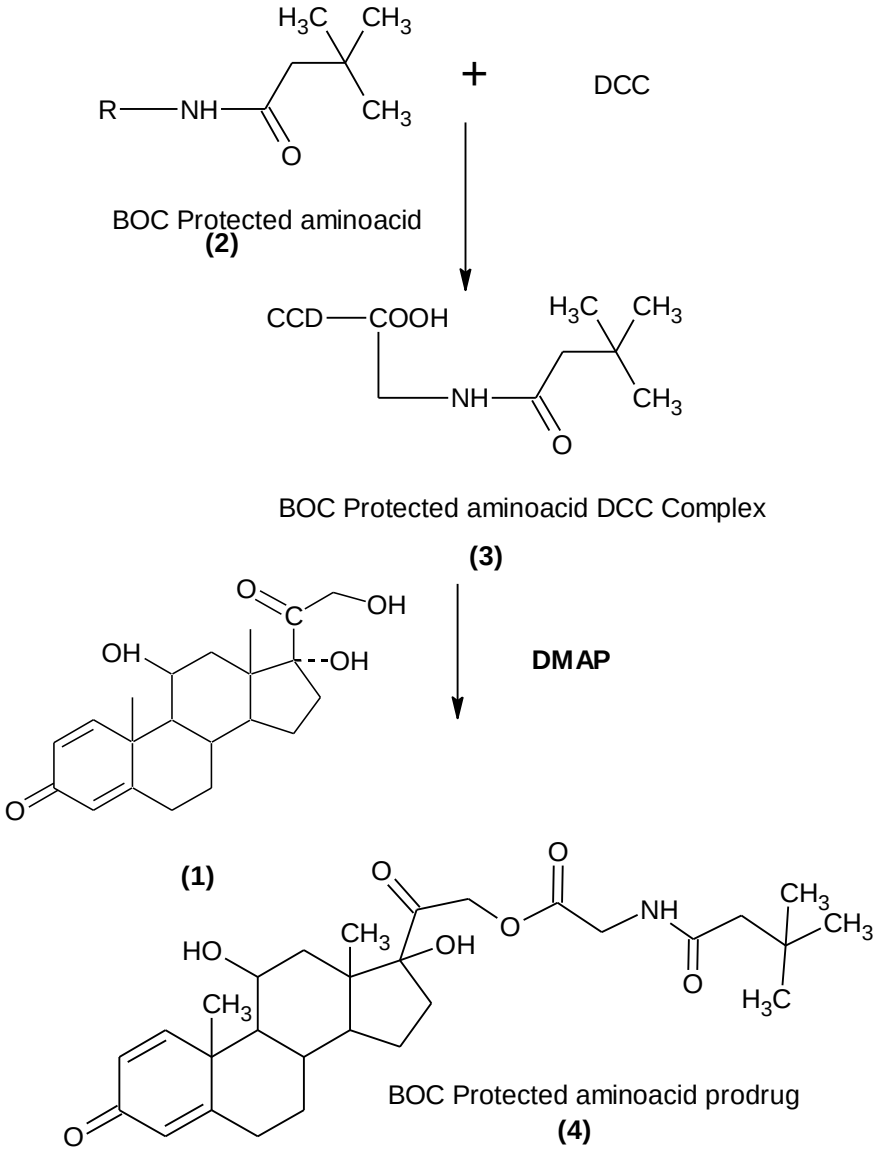
R= 

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminoethnoate)ester (4) – N-BOC Ester

To a 500 ml, 3-necked RBF charged 5.0g (0.0286 mol) of compound 2 and 100 ml of ethylacetate at RT and stirred the reaction mass for 30 min. at room temperature under nitrogen atmosphere. Dicyclohexylcarbodiimide (DCC) (7.02 g, 0.0343mol) was added to the above solution and stirred for 1 hr at RT, solid separated out as BOC-glycine-DCC complex (3).

In a separate flask a solution of prednisolone (10.3g, .0286mol) and ethyl acetate (150 ml) at RT and stirred for 1hr. This solution was then added to the solution of BOC-glycine-DCC complex (3) with simultaneous addition of Dimethylaminopyridine (DMAP) (20 mg catalytic amount). The reaction mass was stirred for 20 hrs at room temperature. The completion of the reaction was monitored by TLC (hexane/ethylacetate 4:6). The solid was filtered and hence discarded. The filtrate was recovered under vacuum at 40-50 ^{o}C to obtain 7.73 g (0.0149 mol) residue as the required compound 4.

REACTION SCHEME



Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminoethnoate)ester (201)

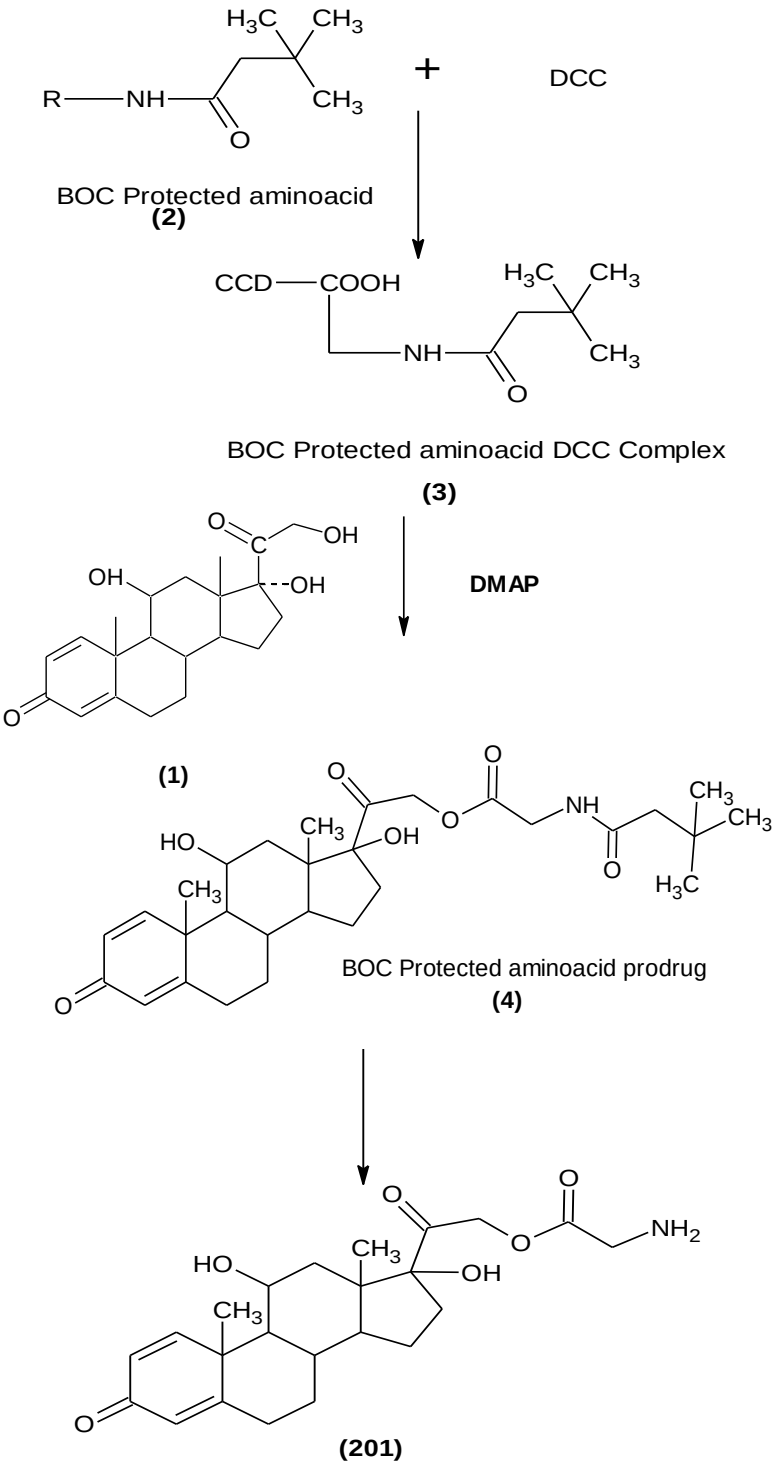
To a 500 ml, 3-necked flask, charged **5.0g (0.009 mol)** of compound **4** and dichloromethane 50 ml at RT. Stirred the reaction mass for 15 min. at RT. Cooled the reaction mass to 0^{o}C and added 50 ml of 1M Aq. Solution of TFA slowly over a period of 30 min at 0 to 5^{o}C. Allowed the reaction mass to come to RT and stirred for 40-50min. Monitored the completion of reaction by TLC (Hexane: Ethlyacetate 6:4). On completion charged 50 ml water and slowly added a solution of NaHC03 to adjust pH neutral. Separated the layers, washed the organic layer first with water (2×100 ml) and then with brine solution. Dried the organic layer over sodium sulphate. Filtered and distilled out the solvent U/Vacc. at 30-35^{o}C to yield required compound 201. This compound was then purified by column chromatography over a column of silica gel and eluting with Hexane:

Ethylacetate in gradually increasing ratio from 9:1 to 6:4. Distillation of the afforded 1.2 g of the required compound **201.**

Yield : 1.2 g (27.2%)

M.Pt. : 160-180°C

REACTION SCHEME 1



Synthesis of Ethyl-N-tert-butyloxycarbonyl-2-aminopropionate (5)

To a 2.0 L, three necked, round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, and two stoppers, charged 145 ml of saturated aq. $NaHCO_3$ solution, 23.0 g (0.393mol) of NaCl, 300 ml of chloroform, 5.0g (0.056 mol) of alanine and stirred for 10 min. at RT. Charged 24.55 g (0.112mol) of BOC anhydride at RT and again stirred for 15 min. Heated the reaction mixture to reflux and maintained u/reflux for 20 hrs under . Cooled the reaction mass to RT and transferred the reaction mass to a separatory funnel and separated the layers. Collected the organic layer and extracted Aq. Layer with chloroform (2 ×100 ml). Combined the organic layers and washed with (2× 100 ml) water. Dried the organic layer over Na_2SO_4, filtered and concentrated U/Vacc. At 40-50 ^oC to afford a total of 9.2g (0.0485 mol) of oil as BOC-protected alanine **(5)**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminopropionate)ester (4) –N-BOC Ester

To a 500 ml, 3-necked RBF charged 5.0g (0.0263 mol) of compound 5 and 100 ml of ethylacetate at RT and stirred the reaction mass for 30 min. at room temperature under nitrogen atmosphere. Dicyclohexylcarbodiimide (DCC) (6.47 g, 0.0316mol) was added to the above solution and stirred for 1 hr at RT, solid separated out as BOC-glycine-DCC complex **(6)**.

In a separate flask a solution of Prednisolone (9.52g, .0263mol) and ethyl acetate (150 ml) at RT and stirred for 1hr. This solution was then added to the solution of BOC-glycine-DCC complex **(6)** with simultaneous addition of Dimethylaminopyridine (DMAP**)** (20 mg catalytic amount). The reaction mass was stirred for 20 hrs at room temperature. The completion of the reaction was monitored by TLC (hexane/ethylacetate 4:6). The solid was filtered and hence discarded. The filtrate was recovered under vacuum at 40-50 ^oC to obtain 7.90 g(0.0148mol) residue as the required compound **7.**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminopropionate)ester (202)

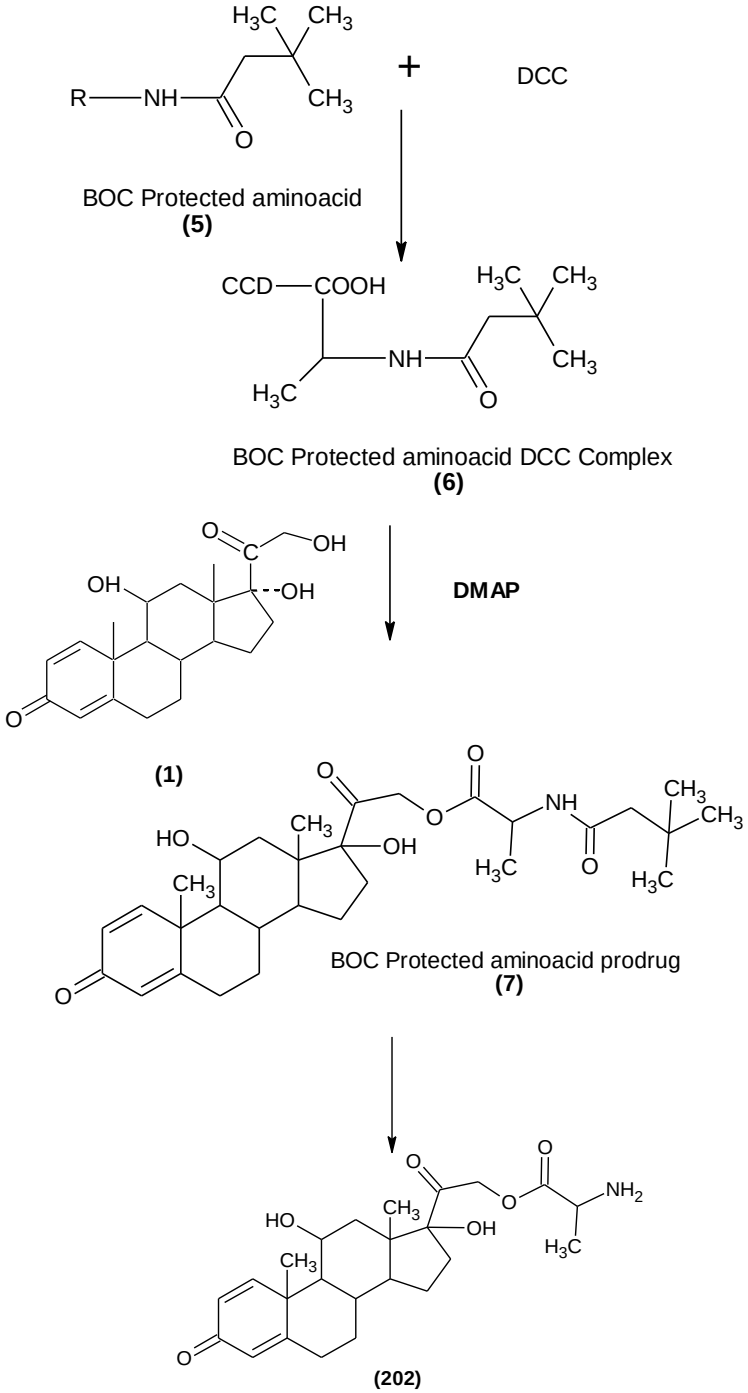
To a 500 ml, 3-necked flask, charged **5.0g (0.0093 mol)** of compound **7** and dichloromethane 50 ml at RT. Stirred the reaction mass for 15 min. at RT. Cooled the reaction mass to 0^oC and added 50 ml of 1M Aq. Solution of TFA slowly over a period of 30 min at 0 to 5^oC. Allowed the reaction mass to come to RT and stirred for 40-50min. Monitored the completion of reaction by TLC (Hexane: Ethylacetate 6:4). On completion charged 50 ml water and slowly added a solution of NaHC03 to adjust pH neutral. Separated the layers, washed the organic layer first with water (2×100 ml) and then with brine solution. Dried the organic layer over sodium sulphate. Filtered and distilled out the solvent U/Vacc. at 30-35^oC to yield required compound 201. This compound

was then purified by column chromatography over a column of silica gel and eluting with Hexane: Ethyl acetate in gradually increasing ratio from 9:1 to 6:4. Distillation of the afforded 1.31 g of the required compound **202.**

Yield : 1.31 g(32.2 %)

M.Pt. : 260-280°C

REACTION SCHEME 2



Synthesis of Ethyl-N-tert-butyloxycarbonyl-2-aminobutanoate (8)

To a 2.0L, three necked, round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, and two stoppers, charged 150 ml of saturated aq. $NaHCO_3$ solution, 17.5 g (0.393mol) of NaCl, 300 ml of chloroform, 5.0g (0.0427 mol) of valine and stirred for 10 min. at RT. Charged 18.68 g (0.0854mol) of BOC anhydride at RT and again stirred for 15 min. Heated the reaction mixture to reflux and maintained u/reflux for 20 hrs under. Cooled the reaction mass to RT and transferred the reaction mass to a separatory funnel and separated the layers. Collected the organic layer and extracted Aq. Layer with chloroform (2 ×100 ml) . Combined the organic layers and washed with (2× 100 ml) water. Dried the organic layer over Na_2SO_4, filtered and concentrated U/Vacc. At 40-50^oC to afford a total of 8.1 g (0.0372 mol) of oil as BOC-protected valine **(8)**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminobutanoate)ester (10) –N-BOC Ester

To a 500 ml, 3-necked RBF charged 5.0g **(0.0229** mol**)** ofcompound **8** and 100 ml of ethylacetate at RT and stirred the reaction mass for 30 min. at room temperature under nitrogen atmosphere. Dicyclohexylcarbodiimide **(DCC) (5.64 g, 0.0316mol)** was added to the above solution and stirred for 1 hr at RT, solid separated out as BOC-glycine-DCC complex **(6)**.In a separate flask a solution of Prednisolone **(8.26 g, .0229mol)** and ethyl acetate (125 ml) at RT and stirred for 1hr. This solution was then added to the solution of BOC-glycine-DCC complex **(6)** with simultaneous addition of Dimethylaminopyridine **(DMAP)** (20 mg catalytic amount). The reaction mass was stirred for 20 hrs at room temperature. The completion of the reaction was monitored by TLC (hexane/ethylacetate 4:6). The solid was filtered and hence discarded. The filtrate was recovered under vacuum at 40-50^oC to obtain 8.26 g (0.0159mol) residue as the required compound **10.**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminobutanoate)ester (203)

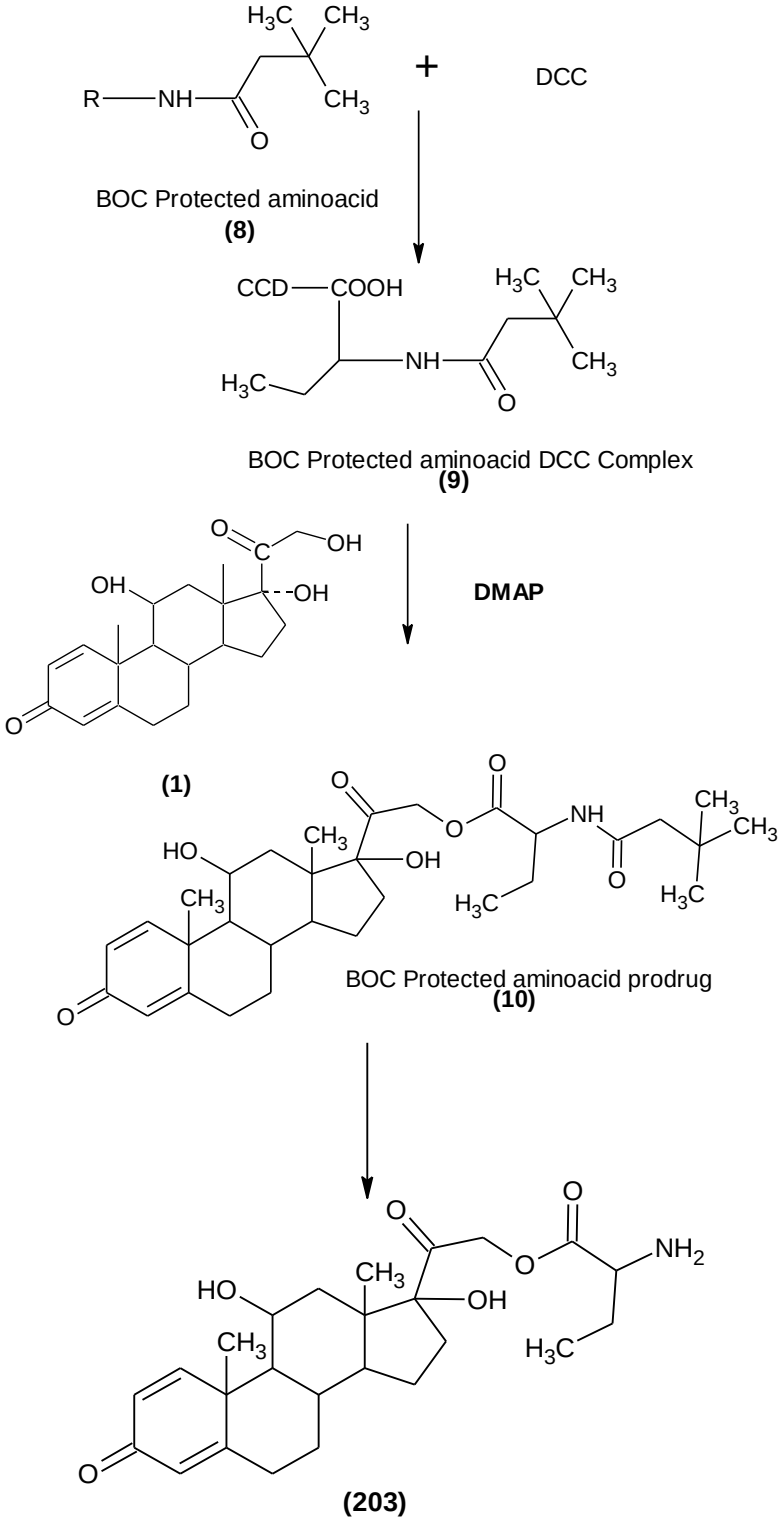
To a 500 ml 3-necked flask, charged **5.0g (0.0089mol)** of compound **10** and dichloromethane 50 ml at RT. Stirred the reaction mass for 15 min. at RT. Cooled the reaction mass to 0^oC and added 50 ml of 1M Aq. Solution of TFA slowly over a period of 30 min at 0 to 5^oC. Allowed the reaction mass to come to RT and stirred for 40-50 min. monitored the completion of reaction by TLC (Hexane: Ethlyacetate 6:4). On completion charged 50 ml water and slowly added a solution of NaHC03 to adjust pH neutral. Separated the layers, washed the organic layer first with water (2×100 ml) and then with brine solution. Dried the organic layer over sodium sulphate. Filtered and distilled out the solvent U/Vacc. at 30-35^oC to yield required compound 201. This compound was then purified by column chromatography over a column of silica gel and eluting with Hexane:

Ethyl acetate in gradually increasing ratio from 9:1 to 6:4. Distillation of the afforded 1.28 g of the required compound **203.**

Yield : 1.28 g (32.16 %)

M.Pt. 220-240°C

REACTION SCHEME 3



Synthesis of Ethyl-N-tert-butyloxycarbonyl-2-amino-3-methylbutanoate (11)

To a 2.0 L, three necked, round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, and two stoppers, charged 150 ml of saturated aq. $NaHCO_3$ solution, 15.62 g (0.269mol) of NaCl, 300 ml of chloroform, 5.0g (0.038 mol) of Leucine and stirred for 10 min. at RT. Charged 16.68 g (0.0768mol) of BOC anhydride at RT and again stirred for 15 min. Heated the reaction mixture to reflux and maintained u/reflux for 20 hrs under. Cooled the reaction mass to RT and transferred the reaction mass to a separatory funnel and separated the layers. Collected the organic layer and extracted Aq. Layer with chloroform (2 ×100 ml). Combined the organic layers and washed with (2× 100 ml) water. Dried the organic layer over Na_2SO_4, filtered and concentrated U/Vacc. At 40-50^oC to afford a total of 6.95 g (0.03mol) of oil as BOC-protected Leucine **(11)**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-amino-3-methylbutanoate)ester (13) –N-BOC Ester.

To a 500 ml, 3-necked RBF charged 5.0g(0.0216mol) of compound 11 and 100 ml of ethylacetate at RT and stirred the reaction mass for 30 min. at room temperature under nitrogen atmosphere. Dicyclohexylcarbodiimide (DCC) (5.3 g, 0.0259mol) was added to the above solution and stirred for 1 hr at RT, solid separated out as BOC-glycine-DCC complex (6). In a separate flask a solution of Prednisolone (7.79g, .0216mol) and ethyl acetate (120ml) at RT and stirred for 1hr. This solution was then added to the solution of BOC-glycine-DCC complex (6) with simultaneous addition of Dimethylaminopyridine (DMAP) (20 mg catalytic amount). The reaction mass was stirred for 20 hrs at room temperature. The completion of the reaction was monitored by TLC (hexane/ethylacetate 4:6). The solid was filtered and hence discarded. The filtrate was recovered under vacuum at 40-50^oC to obtain 8.93 g (0.0155mol) residue as the required compound **13.**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-amino-3-methylbutanoate)ester (204)

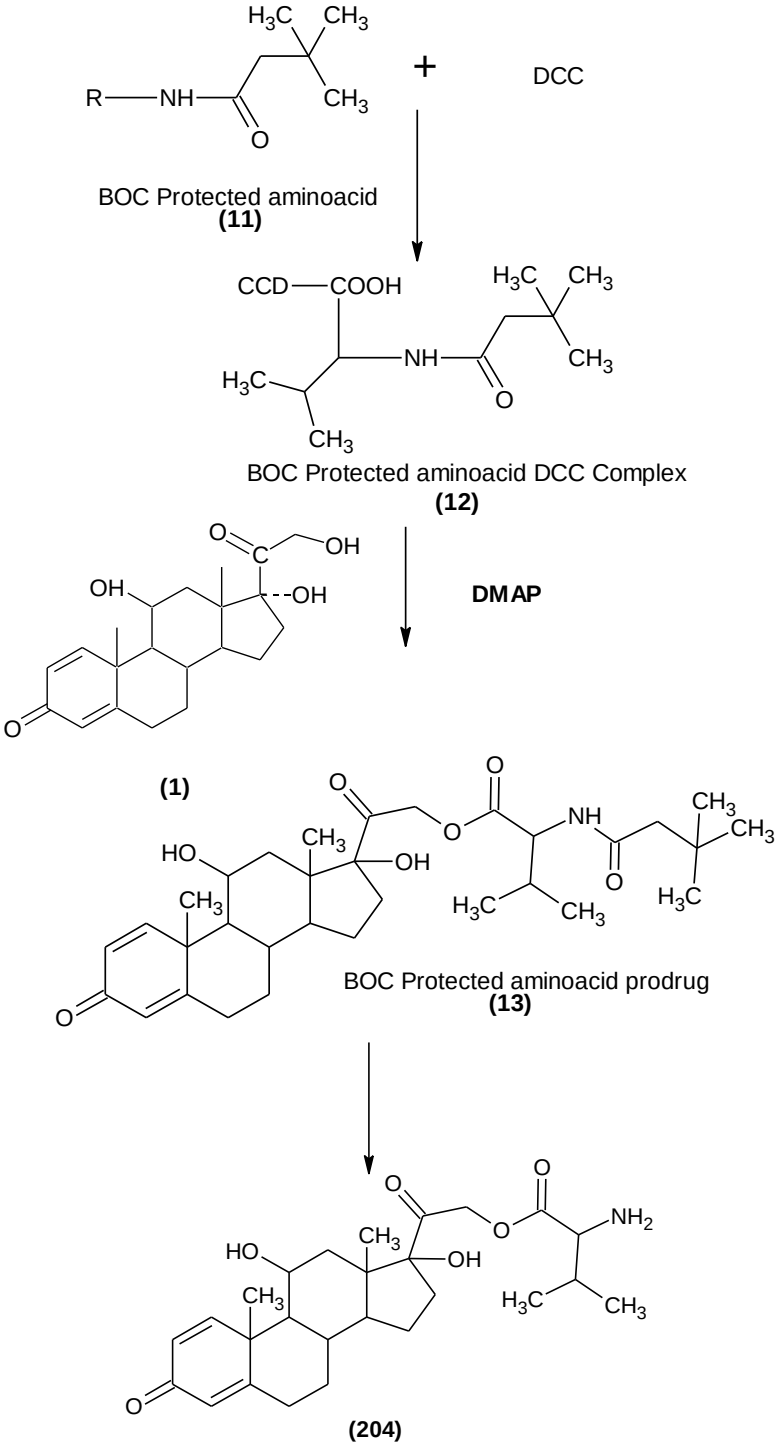
To a 500 ml 3-necked flask, charged 5.0g (0.0087mol) of compound 13 and dichloromethane 50 ml at RT. Stirred the reaction mass for 15 min. at RT. Cooled the reaction mass to 0^oC and added 50 ml of 1M Aq. Solution of TFA slowly over a period of 30 min at 0 to 5^oC. Allowed the reaction mass to come to RT and stirred for 40-50 min. monitored the completion of reaction by TLC (Hexane: Ethlyacetate 6:4). On completion charged 50 ml water and slowly added a solution of NaHC03 to adjust pH neutral. Separated the layers, washed the organic layer first with water (2×100 ml) and then with brine solution. Dried the organic layer over sodium sulphate. Filtered and distilled out the solvent U/Vacc. at 30-35^oC to yield required compound 201. This compound

was then purified by column chromatography over a column of silica gel and eluting with Hexane: Ethylacetate in gradually increasing ratio from 9:1 to 6:4. Distillation of the afforded 1.12g of the required compound **204.**

Yield : 1.12 g (27.9 %)

M.Pt. : 205-225°C

REACTION SCHEME 4



Synthesis of Ethyl-N-tert-butyloxycarbonyl-2-aminophenylacetate (14)

To a 2.0 L, three necked, round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, and two stoppers, charged 150 ml of saturated aq. $NaHCO_3$ solution, 13.55 g (0.231mol) of NaCl, 300 ml of chloroform, 5.0g (0.331 mol) of Phenyl glycine and stirred for 10 min. at RT. Charged 14.47 g (0.0662mol) of BOC anhydride at RT and again stirred for 15 min. Heated the reaction mixture to reflux and maintained u/reflux for 20 hrs under. Cooled the reaction mass to RT and transferred the reaction mass to a separatory funnel and separated the layes. Collected the organic layer and extracted Aq. Layer with chloroform (2 ×100 ml). Combined the organic layers and washed with (2× 100 ml) water. Dried the organic layer over Na_2SO_4, filtered and concentrated U/Vacc. At 40-50^oC to afford a total of 6.17 g (0.0245 mol) of oil as BOC-protected phenyl glycine **(14)**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminphenylacetate)ester (16) –N-BOC Ester

To a 500 ml, 3-necked RBF charged 5.0g (0.0198mol) of compound 14 and 100 ml of ethylacetate at RT and stirred the reaction mass for 30 min. at room temperature under nitrogen atmosphere. Dicyclohexylcarbodiimide (DCC) (5.42 g, 0.0248mol) was added to the above solution and stirred for 1 hr at RT, solid separated out as BOC- phenyl glycine -DCC complex **(16)**.In a separate flask a solution of Prednisolone (6.79 g, 0.0188mol) and ethyl acetate (125 ml) at RT and stirred for 1hr. This solution was then added to the solution of BOC-glycine-DCC complex (6) with simultaneous addition of Dimethylaminopyridine (DMAP) (20 mg catalytic amount). The reaction mass was stirred for 20 hrs at room temperature. The completion of the reaction was monitored by TLC (hexane/ethylacetate 4:6). The solid was filtered and hence discarded. The filtrate was recovered under vacuum at 40-50^oC to obtain 7.44 g (0.0125mol) residue as the required compound **16.**

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminophenylacetate)ester (205)

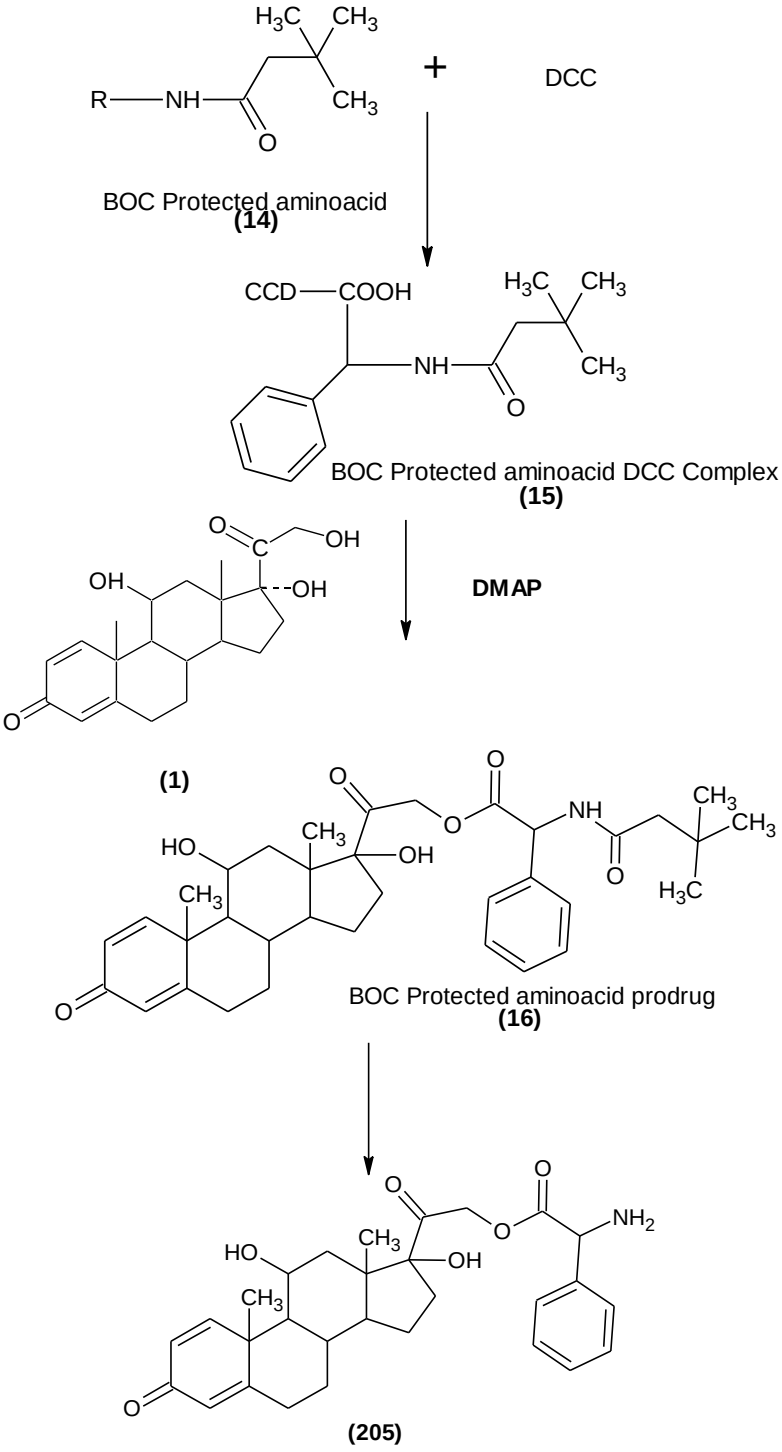
To a 500 ml 3-necked flask, charged 5.0g (0.0084mol) of compound 10and dichloromethane 50 ml at RT. Stirred the reaction mass for 15 min. at RT. Cooled the reaction mass to 0^oC and added 50 ml of 1M Aq. Solution of TFA slowly over a period of 30 min at 0 to 5^oC. Allowed the reaction mass to come to RT and stirred for 40-50 min. Monitored the completion of reaction by TLC (Hexane: Ethlyacetate 6:4). On completion charged 50 ml water and slowly added a solution of NaHC03 to adjust pH neutral. Separated the layers, washed the organic layer first with water (2×100 ml) and then with brine solution. Dried the organic layer over sodium sulphate. Filtered and distilled out the solvent U/Vacc. at 30-35^oC to yield required compound 201. This compound

was then purified by column chromatography over a column of silica gel and eluting with Hexane: Ethylacetate in gradually increasing ratio from 9:1 to 6:4. Distillation of the afforded 1.26g of the required compound **205.**

Yield : 1.26 g (31 %)

M.Pt. 218-250°C

REACTION SCHEME 5



SPECTRAL DATA

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminoethnoate)ester (201)

IR (KBr) : 3434, 3357, 3217, 3034, 2920, 1713, 1621, 1591, 1493, 1470, 1359, 1293, 1170, 996, 774, 691cm^{-1}

^{1}HNMR (DMSO) δ0.87 s (3H), 0.95-1.16 m(2H), 1.30-1.40m (1H),1.45s(3H),1.50-1.60 m (2H), 1.61-1.80 m(2H), 1.92-1.99 dd (1H), 2.0-2.15 m (2H), 2.30-2.35m(1H),2.55-2.7m(2H), 3.55 s (2H), 3.7 s (2H), 4.15 s (1H), 4.17-4.25d(1H),4.40 s(1H), 4.60-4.65d(1H), 4.9s(1H), 5.90s(1H), 6.15-6.20 d(1H), 7.35 d(1H)

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminopropionate)ester (202)

IR (KBr): 3434,3085, 2868, 1742, 1516, 1360, 1345, 1293, 1203, 1095, 1076, 924, 810, 682cm^{-1}

^{1}HNMR (DMSO) : δ0.90 s (3H), 0.95-1.05d(3H), 0.95-1.05m(1H),1.1-1.15 m(1H), 1.35-1.40m (1H),1.45s(3H),1.50-1.65 m (2H), 1.70-1.80 m(2H), 1.90-2.0 dd (1H), 2.05-2.15 m (2H), 2.25-2.35m(1H),2.55-2.7m(2H), 2.75-2.90 m (1H), 3.5 s (2H), 4.15 s (1H), 4.15-4.25d(1H),4.35 s(1H), 4.60-4.65d(1H), 4.9s(1H), 5.90s(1H), 6.20 d(1H), 7.35 d(1H).

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminobutanoate)ester (203)

IR (KBr) : 3467, 2936, 1718, 1655, 1594, 1504, 1462, 1416, 1336, 1228, 1128, 1042 cm^{-1}

^{1}HNMR (DMSO) : δ0.85 s (3H), 0.95t(3H), 0.95-1.05m(2H), 1.1-1.15 m(1H), 1.35-1.40m (1H), 1.45s(3H),1.50-1.62 m (2H), 1.70-1.80 m(2H), 1.95-2.0 dd (1H), 2.05-2.15 m (2H), 2.28-2.35m(1H), 2.55-2.7m(2H), 2.75-2.90 m (1H), 3.5 s (2H), 3.65m(1H), 4.1 s (1H), 4.15-4.23d(1H), 4.40 s(1H), 4.60-4.65d(1H), 4.9s(1H), 5.95s(1H), 6.15-6.20 d(1H), 7.35 d(1H)

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-amino-3-methylbutanoate)ester (204)

IR (KBr) : 3388, 2915, 1706, 1690, 1655, 1615, 1407, 1369, 1304, 1132, 1039, 901, 825 cm^{-1}

^{1}HNMR (DMSO) : δ0.85 s (3H), 0.95t(3H), 1.0-1.05m(2H), 1.1-1.2 m(1H), 1.30d(6H), 1.3-1.40m (1H), 1.45s(3H),1.45-1.60 m (2H), 1.70-1.78 m(2H), 1.90-2.0 dd (1H), 2.05-2.15 m (2H), 2.30-2.35m(1H), 2.55-2.7m(2H), 3.5 s (2H), 3.78-3.82 m(1H), 4.1 s (1H), 4.15-4.25d(1H), 4.38 s(1H), 4.60-4.65d(1H), 4.9s(1H), 5.95s(1H), 6.18-6.22 d(1H), 7.35 d(1H).

Synthesis of 11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-aminophenylacetate)ester (205)

IR (KBr): 3485, 3390, 2974, 2939, 1729, 1657, 1608, 1160, 885 cm^{-1}

^{1}HNMR (DMSO) : δ0.88 s (3H), 0.95-1.05m(2H), 1.08-1.15 m(1H), 1.35-1.40m (1H), 1.45s(3H),1.50-1.60 m (2H), 1.68-1.78 m(2H), 1.95-2.0 dd (1H), 2.0-2.15 m (2H), 2.3-2.35m(1H), 2.55-2.68m(2H), 3.55s (2H), 4.1 s (1H), 4.15-4.25d(1H), 4.40 s(1H),4.5-4.55 m(1H), 4.60-4.65d(1H), 4.9s(1H), 5.95s(1H), 6.15-6.20 d(1H), 7-7.05d(2H), 7.1-7.2dd (1H),7.2-7.3dd(2H), 7.35d (1H)

Table 1: Physical Data of Synthesized Compounds (201-205)

Compound	R	Molecular formula	Molecular Weight	Melting Point (°C)	Yield (%)
201	–CO–CH_2–NH_2	$C_{23}H_{31}O_6N$	419	160-180	27.2
202	–CO–CH(–CH_3)–NH_2	$C_{24}H_{33}O_6N$	433	260-280	32.2
203	–CO–CH(–CH_2–CH_3)–NH_2	$C_{25}H_{35}O_6N$	447	220-240	32.16
204	–CO–CH(–CH(CH_3)–CH_3)–NH_2	$C_{26}H_{35}O_6N$	461	205-225	27.9
205	–CO–CH(–C$_6$H$_5$)–NH_2	$C_{29}H_{35}O_6N$	495	218-250	31

Pharmacological activity:

Adult albino rats weighing (150-200g) of either sex were used as experimental animals. All the animals were housed in groups of 4-6 per cage at a temperature of 25± 1 ºC and a relative humidity of 45-55%. A 12 hr dark and 12 hr light cycle was followed during the experiments. Animals were allowed free access to food and water ad libitum. During the study period, guidelines of Committee for the purpose of Control and Supervision of Experiments on animals (CPCSEA), (Regd No. 1181/PO/Ebi/08/CPCSEA) Institutional Animal Ethics Committee (IAEC) were followed for the maintenance of animals.

Anti-inflammatory activity by Carrageenan- induced rat paws edema method

In the present study, anti-inflammatory activity was determined in albino rats of either sex according to the method by Winter et al., (1962) [17]. All drugs were given orally to the respective

groups as a suspension in 15% DMSO one hour before carrageenan injection. The procedure followed was, acute inflammation produced by injection of carrageenan (0.1 ml of 1% w/v suspension)[18], in the right hind paw of the rats under the plantar aponeurosis. It was injected +1h after the oral administration of the drug. The paw edema volume was measured with the help of plethysmograph by mercury displacement method at 0 h and 3h (immediately after injection and 3h post injection of carrageenan). The % edema is shown in Table 1. The percent anti-inflammatory activity was calculated according to the formula as given below:

Percentage inhibition of paw edema = $(1–V_t/V_c) \times 100$

Where V_c represent average increase in paw volume (average inflammation) of the control group of rats at a given time; and V_t was the average inflammation of the test compound treated rats at the same time.

Statistical Analysis:

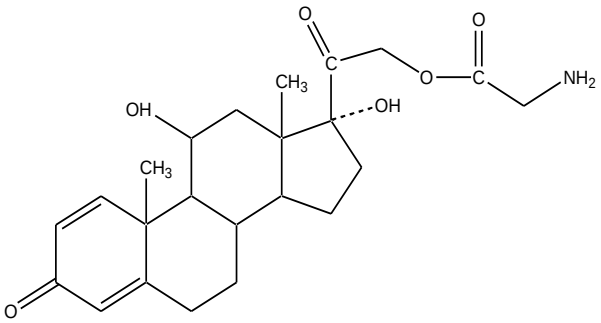
Statistical analysis of the biological activity of synthesized compounds on animals was performed by one-way variance (ANOVA) followed by Tukey's test; as "p" value of less than 0.05 was considered as statistically significant. All values were expressed as mean ± SEM. The SIGMA Plot 13 was used for statistical analysis.

RESULTS AND DISUCSSION

Chemistry

In the present study total five novel compounds were synthesized and evaluated for their pharmacological activities. Synthesis of these compounds was done as indicated in scheme 1 to 5.

(201)

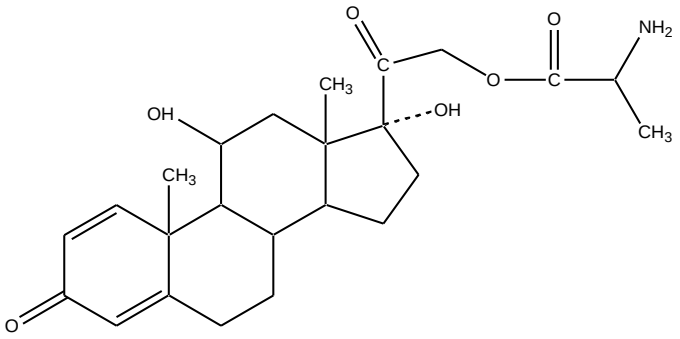


11β,17α-Dihydroxypregna-1,4 diene- 3,20-dione-21(2-oxo-2-aminoacetate)ester(201)

This compound was prepared by reacting BOC-protected Glycine **(2)** with DCC in ethylacetate at RT for 30 min. to form BOC-glycine-DCC complex **(3)**.This complex was then allowed to react with Prednisolone **(1)** in the presence of DMAP to form the protected amino acid ester **(4)** which on deprotection afforded the required amino acid ester **(201).** The amino acidester **201** in I.R

spectrum showed characteristic vibrational bands viz **3434- 3217** cm^{-1} corresponding to –OH and NH- str., Strong band at **1700 to 1690** cm^{-1} corresponding to C=O str.of Ester group, medium band at **1293** cm^{-1} corresponding to C-O str.

In ^{1}HNMR spectra two singlet's at **δ4.1 and δ4.9** corresponding to 2H of NH_2 confirms the presence of **– CH_2-NH_2** in the ester moiety and the presence of a singlet at **δ3.65** corresponding to **2H of –CH_2** group in side chain.

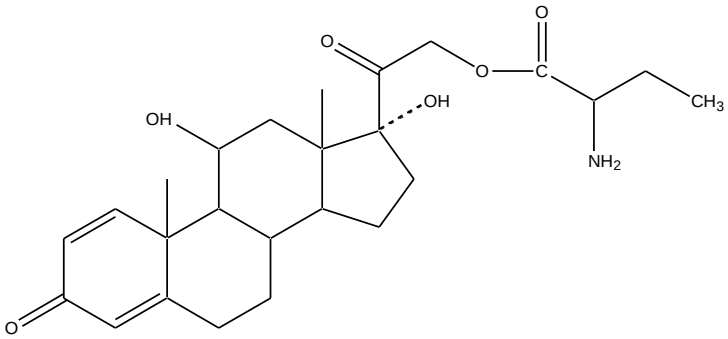


(202)

11β, 17*α*-Dihydroxypregna-1,4diene-3,20-dione-21-(2-oxo-2-aminopropanoate) ester (202)

This compound was prepared by reacting BOC-protected Alanine **(5)** with DCC in ethylacetate at RT for 30 min. to form BOCalanine-DCC complex **(6)**. This complex was then allowed to react with Prednisolone **(1)** in the presence of DMAP to form the protected alanine ester of Prednisolone **(7)** which on deprotection afforded the required aminoacid ester **(202)** .

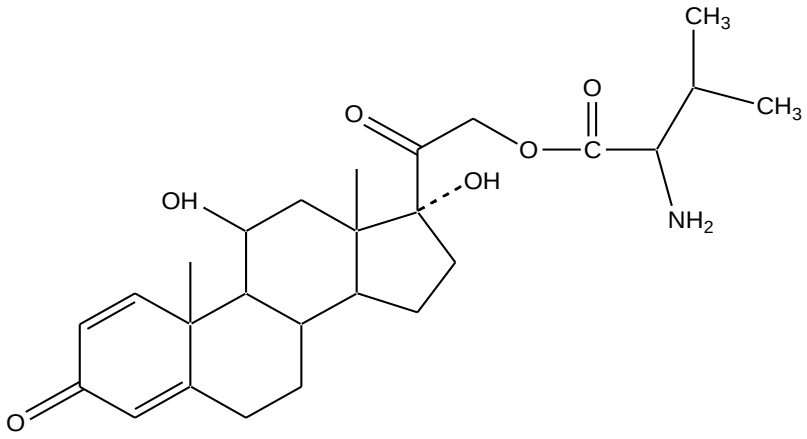
The aminoacidester **202** in I.R spectrum showed characteristic vibrational bands in the region of **3500-3300** cm^{-1} corresponding to –OH and NH- str, Stre.bands at **1742 to 1690** cm^{-1} corresponding to C=O str.of Ester group, medium band at **1293** cm^{-1} corresponding to C-O str. In^{1}HNMR spectra a dublet corresponding to **3H** at **δ 1.1** confirms the presence of **–CH_3-CH** group attached to the **C=O** group, two singlets at **δ4.1 and δ4.9** corresponding to**2H** of **NH_2**group confirms the presence **of NH_2**group and presence of a multiplet at **δ2.75-2.9** corresponding to**1H** confirms thepresence of **–CH .**



(203)

11β, 17α-Dihydroxypregna-1,4 diene-3,20-dione -21-(2-oxo-2-aminobutanoate)ester (203)

This compound was prepared by reacting BOC-protected valine **(8)** with DCC in ethylacetate at RT for 30 min. to form BOC-Valine-DCC complex **(9)**.This complex was then allowed to react with Prednisolone **(1)** in the presence of DMAP to form the BOC-protected Valine ester of Prednisolone **(10),** which on deprotection afforded the required aminoacid ester **(203).** The aminoacidester **203** in I.R spectrum showed characteristic vibrational bands in the region of 3500-3300 cm^{-1} corresponding to –OH and NH- str, two band at 2750 and 2850 cm^{-1}, Strong band at **1718 to 1655** cm^{-1} corresponding to C=O str. of Ester group, medium band at **1228** cm^{-1} corresponding to C-O str.In ^{1}H-NMR Spectra a triplet at **δ0.97** corresponding to **3H** shows the presence of **–CH_3** , a merged multiplet corresponding to 2H at **δ1.71** confirms the presence of **–CH2** groups, a **1H** multiplet at **δ3.65** shows the presence of **–CH** attached with **NH_2**and two singlet's corresponding to **2H** at **δ4.9** and **δ4.1** for **NH_2** group

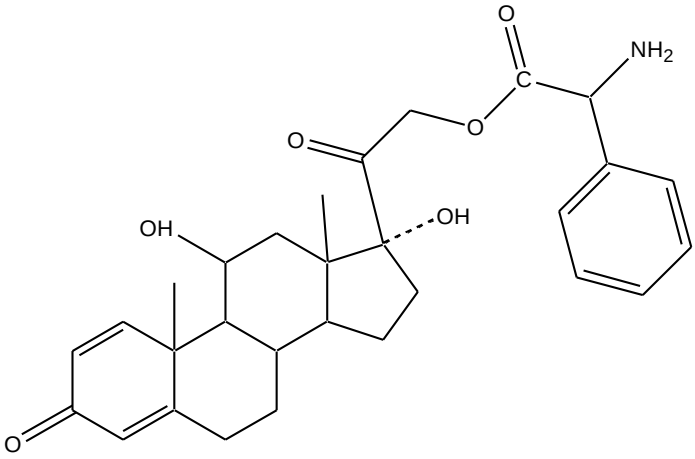


(204)

11β, 17α-Dihydroxypregna-1,4-diene-3,20-dione-21-(2-oxo-2-amino-3-methyl butanoate) ester (204)

This compound was prepared by reacting BOC-protected Leucine **(11)** with DCC in ethylacetate at RT for 30 min. to form BOC-Leucine-DCC complex **(12)**.This complex was then allowed to react with Prednisolone **(1)** in the presence of DMAP to form the BOC protected Leucine ester of Prednisolone **(13)** which on deprotection afforded the required aminoacid ester **(204)** . The amino acidester **204** in I.R spectrum showed characteristic vibrational bands in the region of 3500-3300 cm^{-1} corresponding to –OH and NH- structure. Strong band at **1706 to 1690** cm^{-1} corresponding to C=O str. of Ester group, medium band at 1132 cm^{-1} corresponding to C-O structure. In^{1}HNMR spectra a **doublet** corresponding to **6H** at **δ1.21**confirms the presence of **2CH_3**groups, a multiplet

corresponding to 1H at **δ2.65** confirms iso –**CH** group and a multiplet at **δ3.75** corresponding to **1H** confirms the presence of **–CH** attached with **–NH_2**group



(205)

11β, 17α-Dihydropregna-1,4-diene-3,20-dione-21-(2-oxo-2-aminophenylacetate) ester(205)

This compound was prepared by reacting BOC-protected Phenylglycine **(14)** with DCC in ethylacetate at RT for 30 min. to form BOC-Phenylglycine-DCC complex **(15)**.This complex was then allowed to react with Prednisolone **(1)** in the presence of DMAP to form the BOC protected Phenylglycine ester of Prednisolone **(16)** which on deportation afforded the required amino acid ester **(205).** The amino acid ester **205** in I.R spectrum showed characteristic vibrational bands viz **3500- 3330** cm^{-1} corresponding to –OH and NH- str., Strong band at **1729 to 1700** cm^{-1} corresponding to C=O str. of ester group, medium band at **1160** cm^{-1} corresponding to C-O structure. In ^{1}HNMR spectra the presence of two singlet at **δ5.9** and **δ4.1** corresponding to **2H** confirms the presence of **–NH_2** group and presence of a multiplet corresponding to **$_1$H at δ4.5** confirms the presence of **–CH** group attached with **NH_2** group**,** presence of **three** signals corresponding to **5** aromatic protons in three groups at **δ7.0-7.3** (**7.0d -2H, 7.1d-1H, 7.2d2H,**), confirms the aromatic moiety.

Table 2: Anti-inflammatory activity of tested compounds and Prednisone:

S. No.	Treatments	Dose (mg/kg)	Percentage (%) Edema at 3 hr	Percentage (%) Reduction in Edema at 3 hr
	Control	(1mg/ml)	100±5.25	0.00
	Prednisone(Ref.)	(25 mg/kg)	16.55±1.25 [a]	83.45±3.75 [a]
	201	(25 mg/kg)	59.35±2.05	40.65±1.79
	202	(25 mg/kg)	67.45±2.85	32.55±1.09
	203	(25 mg/kg)	34.50±1.70 [a]	65.50±2.85 [a]
	204	(25 mg/kg)	29.34±1.55 [a]	70.66±3.15 [a]
	205	(25 mg/kg)	27.75±1.65 [a]	72.25±3.25 [a]

Values are mean ± standard error of mean (SEM) (n=6). [a] Statistically significant compared to control group ($p \leq 0.05$). Data was analyzed by unpaired one-way ANOVA test

Anti-inflammatory activity of test compounds:

For the determination of the Anti-inflammatory potency of the compounds, one standard test were realized at 25 mg/kg rat body weight namely, the protection against Carrageenan induced edema according Winter et al. [17]. Regarding the protection against carrageenan induced edema, three compounds namely were found potent when compared with Prednisolone as reference. From the Table 1, it was found that out of 5 compounds of series 201-205, three compounds (205, 204 & 203) showed significant results in comparison with standard prednisolone. Amongst all the compounds showed potent anti-inflammatory activity and rest of the compounds showed moderate or less activity.

DISCUSSION

In this investigation we have demonstrated that out of five amino acid ester derivatives of prednisolone, three derivatives, 205, 204 and 203 reduced the edema (induced by Carrageenan) comparable to prednisolone. Other derivatives, 201, 202 did not show any significant decrease in carrageenan induced edema. The potency of these amino acid ester derivatives compared to prednisolone was lower. The order of potency was prednisolone> 205 >204 >203 >201 >202. From the results it can be illustrated that derivative 205, contain aromatic amino acid ester (2-aminophenylacetate) was most potent as it enhanced the lipophilicity in comparison to other derivatives and hence more passive membrane permeability and more anti-inflammatory effect[14,15]. From the Table 2, it can be illustrated that as the lipophilicity of the amino acid ester derivative decreases, the passive membrane permeability and anti-inflammatory activity decreases. Most of the ester derivatives of prednisolone showed anti-inflammatory activity as compared to the standard drug. So, these types of amino acid derivatives of prednisolone can serve as future therapeutic leads for the discovery of anti-inflammatory drugs.In conclusion, the pharmacological activity shown by the amino acid ester derivatives of prednisolone offer the possibility of being used as novel therapeutic anti-inflammatory agent.

CONCLUSION

Selected compounds after synthesized were screened for their in vivo anti-inflammatory activity and found three out of five compounds of 200 series showed significant ($p<0.05$) results as compare to disease control group. The pharmacological activities exhibited by synthesized novel compounds have confirmed that they may serve the purpose of being accepted as the novel

therapeutic agents as anti inflammatory agents. Furthermore, an extensive toxicological study of these derivatives is highly recommended to assess the safety of the derivatives studied.

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REFERENCES

1. Amly W, Karaman R. Recent updates in utilizing prodrugs in drug delivery (2013-2015). Expert Opin Drug Deliv 2016;6:1-21
2. Wermuth CG, Ganellin CR, Lindberg P, Mitscher LA. "Glossary of terms used in medicinal chemistry. Pure Appl Chem 1998;70:1129-43.
3. Valentino JS. Prodrug Strategies for Improving Drug-Like Properties. Borchardt R, Kerns E, Hageman M, Thakker D, Stevens J. (Eds.). Optimizing the "Drug-Like" Properties of Leads in Drug Discovery. Springer, New York, 2006; 221–42.
4. Beaumont K, Webster R, Gardner I, Dack K. Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. Curr Drug Metab 2003;4:461–85.
5. Ettmayer P, Amidon GL, Clement B, Testa B. Lessons learned from marketed and investigational prodrugs. J Med Chem 200;47:2393–404.
6. Mizoi K, Takahashi M, Haba M, Hosokawa M. Synthesis and evaluation of atorvastatin esters as prodrugs metabolically activated by human carboxylesterases.Bioorg Med Chem Lett 2016;26:921-33.
7. Bílková E, Imramovský A, Sedlák M. Recent advances in the design and synthesis of prednisolone and methylprednisolone conjugates.Curr Pharm Des 2011;17:3577-95.
8. Huttunen KM, Raunio H, Rautio J. Prodrugs – from serendipity to rational design. Pharmacol Rev 2011;63:750–71.
9. Sheng Y, Yang X, Pal D, Mitra AK. Prodrug approach to improve absorption of prednisolone.Int J Pharm 2015;487:242-9.
10. Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, et al. Prodrugs: design and clinical applications. Nat Rev Drug Discov 2008;7:255–70.
11. Stella VJ: Prodrugs: some thoughts and current issues. J Pharm Sci 2010;99:4755–65.

12. Testa B. Prodrugs; bridging pharmacodynamic/pharmacokinetic gaps. Curr Opin Chem Biol 2009;13:338–44.
13. Stella VJ. Prodrugs as therapeutics. Expert Opin Ther Pat 2004;14:277–80.
14. Guo ZR, Zhao HY. Challenges and strategies of drug innovation. Yao Xue Xue Bao 2013;48: 1031-40.
15. Taylor MD. Improved passive oral drug delivery via prodrugs. Adv Drug Deliv Rev 1996;19:131–48.
16. Liederer BM. Borchardt RT. Enzymes involved in the bioconversion of ester-based prodrugs. J Pharm Sci 2006;95:1177–95.
17. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med1962;111:544-7.
18. DiMartino MJ, Campbell GK, Wolff CE, Hanna N. The pharmacology of arachidonic acid-induced rat paw edema. Agents Actions1987;21:303-5.