**Supplementary data**

**Pregnane-oximino-alkyl-amino-ether compound as a novel class of TGR5 receptor agonist exhibiting anti-diabetic and anti-dyslipidemic activities**

Jyoti Gupta*a*, Dharmendra P. Singh*a*, Prem C. Verma*a*, Neha Rahuja*b*, Rohit Srivastava*b*, Ishbal Ahmad*b*, Natasha Jaiswal*b*, Harish Kumar*b*, Anand P. Gupta*c*, Varsha Guptac, Anamika Misra*d*, Hari N. Kushwahad, Bhawani Singhd, Sheo K Singhd, Anil K. Dwivedi*c*, Jiaur R. Gayen*c*, Sabyasachi Sanyal*b*, Arvind K. Srivastava*b*, Ram Pratap*a\**, Akhilesh K. Tamrakar*b\**

*aDivision of Medicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow-226031*

*bDivision of Bio-chemistry, CSIR-Central Drug Research Institute, Lucknow-226031*

*cDivision of Pharmaceutics, CSIR-Central Drug Research Institute, Lucknow-226031*

*dDivision of Pharmacokinetics, CSIR-Central Drug Research Institute, Lucknow-226031*

**Running title:** Pregnane-oximino-alkyl-amino-ether as anti-diabetic agent.

**Detail of compounds synthesized**

**3β-Hydroxypregna-5, 16-dien-20-one (6)**

The 3β-hydroxypregna-5,16-dien-20-one acetate (**5**, 16-DPA, 2.0 g, 2.23 mmol) and aq. KOH (2.24 g, 50% solution) in ethanol was refluxed for 5 hr. The solvent was removed and residue taken up in chloroform and washed to give the compound **6**. Yield 1.8 g (80%); m.p. 188oC; FABMS: m/z 316 (M+1); IR (KBr): 1640, 1560, 1220 and 780 cm-1; 1H NMR (200 MHz, CDCl3), δ 6.04 (s, 1H, 16-H), 5.36 (d, 1H, 6-H, *J*= 4.02 Hz), 3.58-3.48 (m, 1H, 3-H), 2.21 (s, 3H, 21-CH3), 2.03 (s, 3H, 19-CH3), 0.70 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5, 16-dien-20-one- oxime (7)**

Compound **6** (5 g, 0.014 mole) and hydroxylamine hydrochloride (113 g, 0.016 mole) in dry pyridine (100 ml) was stirred until the clear solution appears. It was then left in tightly closed container in refrigerator for four days, poured into water (500 ml) and separated solid collected by filtration, washed with water and dried to afford compound **7**. Yield 5.0 g (96%); m.p. 210-212oC; FABMS: m/z 330 (M+1); IR (KBr): 3391, 2935, 2366, 1595, 1354, 1055 cm-1; 1H NMR (200 MHz, CDCl3), δ 6.04 (s, 1H, 16-H), 5.35 (d, 1H, 6-H, *J*= 3.6 Hz), 3.56-3.53 (m, 1H, 3-H), 1.89 (s, 3H, 21-CH3), 1.01 (s, 3H, 19-CH3), 0.65 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-ene-20-one-acetate** **(8)**

In parr hydrogenation bottle 3β-hydroxy pregna-5, 16-dien-20-one acetate (**5**,16-DPA, 1.0 g, 2.8 mmol), 10% Pd-C (100 mg) in dry ethyl acetate was shaken in hydrogen atmospheric 5-10 lb pressure for 20 min. After the reaction, the catalyst was filtered through celite and solvent removed in vaccuo to afford compound **8**. Yield 1.0 g (99.4%); m.p. 145oC; FABMS: m/z 358 (M+1); IR (KBr): 3440.6, 2939.4, 1727.9, 1706.3, 1358, 1035 cm-1; 1H NMR (200 MHz, CDCl3): δ 5.36 (d, 1H, *J*= 6.0 Hz), 4.62 – 3.53 (m, 1H), 2.11 (s, 3H, CH3CO), 1.63 (s, 3H, 21-CH3), 1.02 (s, 3H, 19-CH3), 0.67 (s, 3H, 18-CH3).

**3β-Hydroxypregna-5-en-20-one (9)**

The compound **8** (2.0 g, 2.23 mmol) and aq KOH (2.24 g, 50% solution) in ethanol was refluxed for 5 hr. The solvent was removed and residue taken up in chloroform and washed to give the compound **9**. Yield 1.9 g (83.9 %); M.P. 188oC; FABMS: m/z (M+1); IR (KBr): 3433.3, 2930.9, 1688.1, 1539.3, 1355 and 1058.1 cm-1; 1H NMR (200 MHz, CDCl3), δ 5.36 (d, 1H, 6-H, *J*= 4.02 Hz), 3.58-3.48 (m, 1H, 3-H), 2.21 (s, 3H, 21-CH3), 2.03 (s, 3H, 19-CH3), 0.70 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-oxime (10)**

Compound **9** (5 g, 0.014 mole) and hydroxylamine hydrochloride (113 g, 0.016 mole) in dry pyridine (100 ml) was stirred until the clear solution appears. It was then left in tightly closed container in refrigerator for four days, poured into water (500 ml) and separated solid collected by filtration, washed with water and dried to afford compound **10**. Yield 5.0 g (96%); m.p. 200oC; FABMS: m/z 332 (M+1); IR (KBr): 3274, 2828, 1595, 1439, 1367, 1058, 774 cm-1; 1H NMR (200 MHz, CDCl3), δ 5.36 (d, 1H, 6-H, *J*= 4.0 Hz), 3.56-3.51 (m, 1H, 3-H), 1.89 (s, 3H, 21-CH3), 1.01 (s, 3H, 19-CH3), 0.65 (s, 3H, 18-CH3).

**3β-Hydroxypregna-5, 16-dien-20-one-O-(2-piperidinyl-ethyl)-oxime (11a)**

The suspension of 3β-hydroxy-pregna-5,16-dien-20-one-O-oxime (**7**,0.6 g, 1.8 mmol) and NaH (0.2 g, 20 mmol) was stirred for 1 hr at 0oC in DMF. 1-(2-chloro-ethyl)-piperidine hydrochloride (0.33 g, 2 mmol) was added thereafter and the reaction mixture left stirring for another 6 hr at 35oC. DMF was removed under *vaccum* and the crude product thus obtained was purified by column chromatography to afford compound **11a**; yield 0.6 g (68%); m.p. 135-138oC; FABMS: m/z 441 (M+1); 1H NMR (200 MHz, CDCl3): δ 6.00 (s, 1H, 16-H), 5.37 (d, 1H, 6-H, *J*= 2.0 Hz), 4.23 (t, 2H, OCH2), 3.48 (m, 1H, 3-H), 2.67 (t, 2H, NCH2), 2.48 (t, 4H, NCH2), 1.93 (s, 3H, 21-CH3), 1.489-1.25 (m, 6H, CH2), 1.05 (s, 3H, 19-CH3), 0.96 (s, 3H, 18-CH3).

**3*β*-Hydroxypregna-5, 16-dien-20-one-O-(2-azepan-1-yl-ethyl)-oxime (11b)**

The suspension of 3β-hydroxy-pregna-5,16-dien-20-one-oxime (**7)**,1-(2-chloro-ethyl)-azepine hydrochloride in presence of NaH was reacted as above to afford compound **11b**, yield 0.6 g (68%); m.p. 127-130oC; FABMS: m/z 455 (M+1); 1H NMR (200 MHz, CDCl3): δ 6.00 (s, 1H, 16-H), 5.37 (d, 1H, 6-H, *J*= 2.0 Hz), 4.23 (t, 2H, OCH2), 3.48 (m, 1H, 3-H), 2.67 (t, 2H, NCH2), 2.48 (t, 4H, NCH2), 1.93 (s, 3H, 21-CH3), 1.489-1.25 (m, 6H, CH2), 1.05 (s, 3H, 19-CH3), 0.96 (s, 3H, 18-CH3).

**3*β*-Hydroxypregna-5, 16-dien-20-one-O-(2-morpholin-4-yl-ethyl)-oxime (11c)**

The suspension of 3β-hydroxy-pregna-5-16-dien-20-one-oxime (**7**),1-(2-chloro-ethyl)-morpholine hydrochloride in presence of NaH was reacted as above to afford compound **11c**, yield 0.5 g (67%); m.p. 132-135oC; FABMS: m/z 444 (M+1); 1H NMR (200 MHz, CDCl3), δ 6.04 (s, 1H, 16-H), 5.37 (d, 1H, 6-H, *J*= 4.0 Hz), 4.23 (t, 2H, OCH2), 3.73 (t, 4H, NCH2), 2.71 (t, 2H, NCH2), 2.55 (t, 4H, NCH2), 1.93 (s, 3H, 21-CH3), 1.00 (s, 3H, 19-CH3), 0.96 (s, 3H, 18-CH3).

**3*β*-Hydroxypregna-5, 16-dien-20-one-O-(2-diethylamino-ethyl)-oxime (11d)**

The suspension of 3β-hydroxy-pregna-5-16-dien-20-one-oxime (**7**),1-(2-chloro-ethyl)-diethylamine hydrochloride in presence of NaH (0.2 g, 20 mmol) was reacted as above to afford compound **11d**, yield 0.62 g (69%); m.p. 156-160oC; FABMS: m/z 429 (M+1); 1H NMR (200 MHz, CDCl3), δ 6.00 (s, 1H, 16-H), 5.36 (s, 1H, 6-H), 4.19 (t, 2H, OCH2), 3.31 (m, 1H, 3-H), 2.80 (t, 2H, NCH2), 2.65-2.58 (q, 6H, NCH2CH3), 1.93 (s, 3H, 21-CH3), 1.05 (s, 3H, 19-CH3), 0.63 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-(2-pyrrolidin-1-yl-ethyl)-oxime (14a)**

The suspension of 3β-hydroxypregna-5-en-20-one-oxime (**10**, 0.6 g, 1.8 mmol) and NaH (0.2g, 20 mmol) in DMF was stirred for 1 hr at 0oC. 1-(2-chloro-ethyl)-pyrrolidine hydrochloride (0.33 g, 2 mmol) was added there after and the reaction mixture left stirring for another 6 hr at 35oC. DMF was removed under *vaccum* and the crude product thus obtained was purified by column chromatography to afford compound **14a**,yield 0.6 g (68%); m.p. 140-142oC; FABMS: m/z 429 (M+1); 1H NMR (200 MHz, CDCl3): δ 5.36 (d, 1H, 6-H, *J*= 3.6 Hz), 4.28 (t, 2H, OCH2), 3.53-3.48 (m, 1H, 3-H), 2.96 (t, 2H, NCH2), 1.81 (s, 3H, 21-CH3), 1.29-1.20 (q, 4H, CH2), 1.01 (s, 3H, 19-CH3), 0.62 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-(2-piperidin-1-yl-ethyl)-oxime (14b)**

The suspension of 3β-hydroxy-pregna-5-en-20-one-oxime (**10**,0.6 g, 1.8 mmol), 1-(2-Chloro-ethyl)-piperidine hydrochloride (0.33 g, 2 mmol) in presence of NaH (0.2 g, 20 mmol) was reacted as above to afford compound **14b**; yield 0.5 g (67 %); m.p. 130-135oC; FABMS: m/z 443 (M+1); 1H NMR (200 MHz, CDCl3): δ 5.36 (d, 1H, 6-H, *J*= 6.0 Hz), 4.18 (t, 2H, OCH2), 3.50-3.49 (m, 1H, 3-H), 2.48 (t, 2H, NCH2), 1.81 (s, 3H, 21-CH3), 1.67-1.42 (m, 4H, CH2), 1.01 (s, 3H, 19-CH3), 0.62 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-(2, 3-epoxypropyl)-oxime (12)**

Suspension of compound **10** (7.0 g, 21.0 mmol), NaH (2.04 g, 85 mmol) in dry DMF (100 ml) was stirred for an hour at 0°C and epichlorohydrine (6.0 ml, 126 mmol) was then added drop wise. The reaction mixture was stirred over night at room temp. DMF removed under vacuum. Residue was extracted with chloroform and washed with water, dried over Na2SO4 and solvent removed in *vaccuo* and the crude was purified by column chromatography to afford compound **12**;yield 6.2 g (75.7%); m.p. 110oC; FABMS: m/z 388 (M+1); 1H NMR (200 MHz, CDCl3), δ 5.37 (d, 1H, 6-H, *J*= 4.32 Hz), 4.35 (dd, 1H, *J*= 11.1 Hz and 4.2 Hz), 4.19 (dd, 1H, *J*= 12.75 and 6.6 Hz), 3.66-3.63 (m, 1H, -CH-OH), 3.42-3.29 (m, 1H), 2.97 (t, 1H, *J*= 6.0 Hz), 2.81-2.76 (m, 1H), 1.97 (s, 3H, 21-CH3), 1.04 (s, 3H, 19-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-(2-hydroxy-3-*iso*-propylamino-propyl)-oxime (13a)**

The reaction of 3β-hydroxy-pregna-5-en-2-one-O-(2,3-epoxypropyl)-oxime (**12**,0.5 g, 1.3 mmol) and *iso*-propylamine (0.33 ml, 3.2 mmol) in dry methanol (50 ml) under reflux as above furnished **13a**, yield 0.5 g (54.3%); m.p. (hygroscopic); FABMS: m/z 447 (M+1); 1H NMR (200 MHz, CDCl3) δ 5.36 (d, 1H, 6-H, *J*= 4.4 Hz), 4.12 (d, 2H, OCH2, *J*= 4.84 Hz), 3.94-3.88 (m, 1H, -CHOH-), 3.55-3.31 (m, 1H, 3-H), 2.89-2.75 (m, 3H, CH2-NH-CH), 1.95 (s, 3H, 21-CH3), 1.42-1.34 (m, 6H, CH{CH3}2), 1.04 (s, 3H, 19-CH3), 0.95 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-[2-hydroxy-3-(4-phenyl-piperazin-1-yl)-propyl]-oxime (13b)**

The reaction of 3β-hydroxy-pregna-5-en-20-one-O-(2,3-epoxypropyl)-oxime (**12**,700 mg, 1.8 mmol) and 4-N-phenylpiperazine (0.5 ml, 3.6 mmol) in dry methanol (40ml) as above afforded **13b**;yield 0.9 g (51%); M.P. 180oC; FABMS: m/z 550 (M+1); 1H NMR (200 MHz, CDCl3) δ 7.30-7.23 (m, 2H, ArH), 6.94-6.82 (m, 3H, Ar-H), 5.36 (d, 1H, 6-H, *J*= 4.0 Hz), 4.09-4.08 (m, 3H, =N-OCH2-CH-OH), 3.52-3.49 (m, 1H, 3-H), 3.21-3.19 (m, 4H, Ar-NCH2), 2.95-2.86 (m, 2H, N-CH2), 2.65-2.59 (m, 4H, NCH2), 1.85 (s, 3H, 21-CH3), 1.01 (s, 3H, 19-CH3), 0.64 (s, 3H, 19-CH3).

**3β-Hydroxy-pregna-5-en-2-one-O-[2-hydroxy-3-pipridinyl-propyl)-oxime (13c)**

The reaction of 3β-hydroxy-pregna-5-en-20-one-O-(2,3-epoxypropyl)-oxime (**12**, 1.0 g, 2.5 mmol) and cyclohexyl amine (0.6 ml, 5.0 mmol) as above afforded compound **13c**; yield 0.7 g (54.7%); m.p. 110oC; FABMS: m/z 473 (M+1); 1H NMR (200 MHz, CDCl3): δ 5.35 (d, 1H, 6-H, *J*= 6.0 Hz), 4.67 (s, 3H, OCH2CHOH), 3.34-3.60 (m, 1H, 3-H), 2.96-2.93 (m, 6H, NCH2), 2.02 (t, 4H, NCH2), 1.82 (s, 3H, 21-CH3), 1.54 (t, 6H), 1.04 (s, 3H, 19-CH3), 0.61 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-[2-hydroxy-3-(4-methyl-piperazin-1-yl)-propyl]-oxime (13d)**

The reaction of 3β-hydroxy-pregna-5-en-20-one-O-(2,3-epoxypropyl)-oxime (**12**, 1.0 g, 2.5 mmol) and 4-methyl-piperazine (0.6 ml, 5.0 mmol) as above afforded compound **13d**; yield 0.9 g (51%); m. p. 100oC; FABMS: m/z 488 (M+1); IR (KBr): 3397, 2934, 1460 cm-1; 1H NMR (200 MHz, CDCl3): δ 5.36 (d, 1H, 6-H, *J*= 4.4 Hz), 4.05 (s, 3H, OCH2-CH{OH}), 3.49 (m, 1H, 3-H), 2.45 (t, 8H, NCH2) 2.30 (s, 3H, NCH3), 1.84 (s, 3H, 21-CH3), 1.14 (s, 3H, 19-CH3), 0.62 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-[2-hydroxy-3-diethylamino-propyl)-oxime (13e)**

The reaction of 3β-hydroxy-pregna-5-en-20-one-O-(2, 3-epoxypropyl)-oxime (**12**, 1.0 g, 2.5 mmol) and diethylamine (0.6 ml, 5.0 mmol) as above afforded compound **13e**; yield 0.7 g (54.5 %); m.p. 157-159oC; FABMS: m/z 433 (M+1); 1H NMR (200 MHz, CDCl3): δ 5.36 (d, 1H, 6-H, *J*= 4.0 Hz), 4.23-4.03 (m, 2H, OCH2CHOH), 3.34-3.48 (m, 1H, 3-H), 3.00-2.83 (q, 4H, CH2), 1.84 (s, 3H, 21-CH3), 1.24 (t, 6H, CH3), 1.09 (s, 3H, 19-CH3), 0.62 (s, 3H, 18-CH3).

**3β-Hydroxy-pregna-5-en-20-one-O-[2-hydroxy-3-diisopropylamino-propyl)-oxime (13f)**

The reaction of 3β-hydroxy-pregna-5-en-20-one-O-(2,3-epoxypropyl)-oxime **(12**, 1.0 g, 2.5 mmol) and diisopropyl amine (0.8 ml, 5.0 mmol) in dry methanol (50 ml) as above to afford compound **13f**;yield 0.7 g (53.7 %); m.p. 135-139oC; FABMS: m/z 476 (M+1); 1H NMR (200 MHz, CDCl3): δ 5.35 (d, 1H, 6-H, *J*= 6.0 Hz), 4.22-4.13 (m, 2H, OCH2CHOH), 3.98-3.41 (m, 1H, 3-H), 2.27 (s, 2H, CH), 1.86 (s, 3H, 21-CH3), 1.12 (t, 12H, CH3), 1.04 (s, 3H, 19-CH3), 0.95 (s, 3H, 18-CH3).