European Journal of Medicinal Chemistry 44 (2009) 1215–1222

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of α-amyrin derivatives and their *in vivo* antihyperglycemic activity☆

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ARTICLE INFO

Article history:
Received 17 April 2008
Received in revised form 22 August 2008
Accepted 4 September 2008
Available online 19 September 2008

Keywords:
α-Amyrin acetate
Amyrin ester derivatives
Antihyperglycemic activity
STZ-S model

ABSTRACT

Ficus racemosa belongs to the family of Moraceae and is commonly known as 'Gular' in north India. Bioactivity guided isolation work on the fruits of *F. racemosa* resulted in the identification of antidiabetic active principle, α-amyrin acetate **7**. Compound **7** lowered the blood glucose levels by 18.4 and 17.0% at 5 and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rat (STZ-S) model at the dose of 100 mg/kg body weight. Fifteen novel derivatives viz, **9**–**21**, **24**, **25** of α-amyrin **8** were prepared and their antihyperglycemic activity profile was assessed. The *p*-chlorobenzoic acid derivative **9** and nicotinic acid derivative **14** showed potent antihyperglycemic activity at 100 mg/kg body weight.

1. Introduction

Diabetes mellitus is characterized by elevated plasma glucose concentrations resulting from insufficient insulin production, insulin resistance, or both [1]. The number of cases of non-insulin dependent diabetes mellitus has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity and ageing of populations [2]. In the year 2000, the number of diabetic patients was 151 million and is estimated to rise to 300 million by 2025 [3,4]. The high prevalence and long-term complications of diabetes mellitus have led to an intense search for new oral antihyperglycemic agents from plants used as traditional medicine for diabetes treatment [5]. The use of natural drugs, such as plants and herbal remedies to treat diseases is very common in Asia and developing countries, where the population is linked with the use of traditional medicines, due to their efficiency or due to the costs of the synthetic drugs and/or pharmaceuticals. Moreover, WHO study groups emphasize strongly the optimal, rational uses of traditional and natural indigenous medicines [6].

A large number of crude plant extracts and purified substances from plants have been tested in clinical trials for the treatment of diabetes [7,8]. However, effects with crude extracts remain obscure if the active principles in the extracts are not characterized. Various groups have reported phytochemicals (Fig. 1) with potential antihyperglycemic attributes. Glycyrin **1**, one of the main PPAR-γ ligands of licorice, significantly decreased the blood glucose levels of genetically diabetic KK-Ay mice [9]. Charantin **2**, a steroidal saponin, obtained from *Momordica charantia* is known to have an insulin-like activity, responsible for its hypoglycemic effect [10]. Charantin stimulates the release of insulin and blocks the formation of glucose in the bloodstream. A friedelane-type

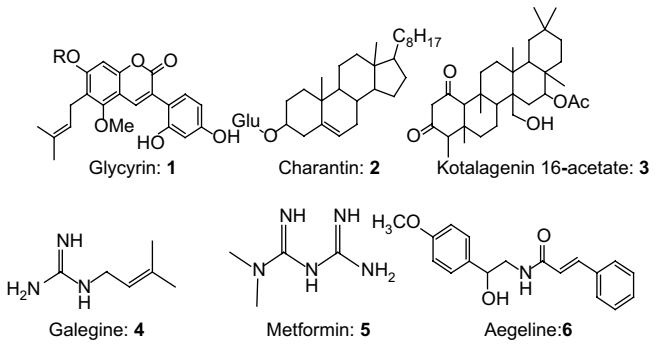

Fig. 1. Naturally occurring antidiabetic agents **1**–**4** and **6**; synthetic antidiabetic agents **5**.

☆ CDRI Comm. No.7334.

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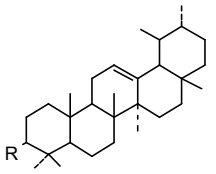
doi:10.1016/j.ejmech.2008.09.011

Table 1
In vivo antihyperglycemic activity of α-amyrin acetate **7** and its synthetic derivatives in STZ-S model



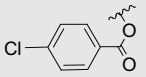
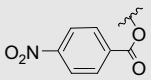
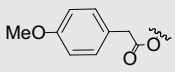
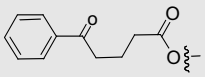
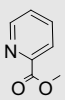
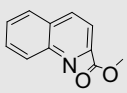
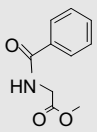
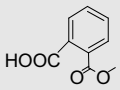
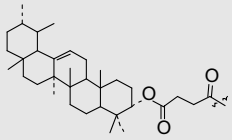
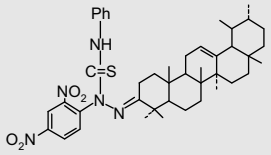
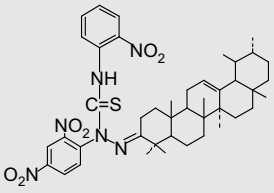
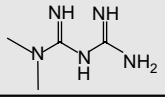
Compound number	R	Dose (mg/kg)	% Antihyperglycemic activity	
			5 h	24 h
7		100	18.4*	17.0*
8	OH	100	13.9*	8.12*
9		100	22.1*	25.6**
10		100	13.8*	27.6**
11		100	17.5*	15.6*
12		100	24.0**	23.5**
13		100	12.7*	22.6*
14		100	18.2*	27.6**
15		100	19.6*	17.8*
16		100	ND	ND
17		100	3.15*	12.1*

Table 1 (*continued*)

Compound number	R	Dose (mg/kg)	% Antihyperglycemic activity	
			5 h	24 h
18		100	24.9**	20.2*
19		100	11.0*	16.5*
20			ND	ND
21		100	24.3**	15.6*
24[a]		100	1.20*,[a]	7.35*,[a]
25			ND	ND
5		100	23.5**	26.5**

ND = Not determined.
*$P < 0.05$ and **$P < 0.01$.
[a] Evaluated in SLM model.

triterpene, kotalagenin 16-acetate **3**, isolated from the roots of *Salacia oblonga* exerted its action as potent α-glucosidase inhibitor [11]. β-Carbolines like harmane, norharmane and pinoline act as potent insulin secretagogues [12]. Metformin **5** is currently used as antidiabetic agent in the treatment of type-2 diabetes. Metformin **5** and its analogues [13,14] were synthesized on the basis of a natural product lead viz, galegine **4**, isolated from the seeds of *Galega officinalis* [5]. Our group recently reported the isolation of the dual acting antihyperglycemic and anti-dyslipidemic agent, aegeline **6**, from the leaves of *Aegle marmelos* [15].

Ficus racemosa Linn. (Moraceae), commonly known as 'Gular', 'Umbar' or 'Jagya-dumbar' [16] is found throughout India. Traditionally, the juice of the leaves is used to treat dysentery, bilious disorders, diarrhoea, diabetes and as a mouth wash [17]. The leaves of the plant showed anti-inflammatory [18] and hepatoprotective [19] activities whilst the bark, root and fruit have been found to possess antidiabetic activity [20,21]. The hypoglycemic potential of leaf extract [22] of the plant was demonstrated in streptozotocin induced diabetic rats whilst glucose lowering efficacy of the stem bark in normal as well as alloxan-induced diabetic rats was also reported [23]. Though the antidiabetic activity of the extract of different parts of the plant is reported in the literature, so far the antihyperglycemic principle has not been identified. Based on the importance of this medicinally valuable herb in the Indigenous system of medicine and its therapeutic importance in ameliorating plasma glucose, bio-assay directed fractionation was carried out in order to localize the antihyperglycemic principle. Since the active principle, α-amyrin acetate was an ester, we prepared several non-natural ester and metformin related derivatives of deacetylated α-amyrin to develop SAR and improve the potency of the lead (Table 1).

2. Results and discussion

2.1. Chemistry

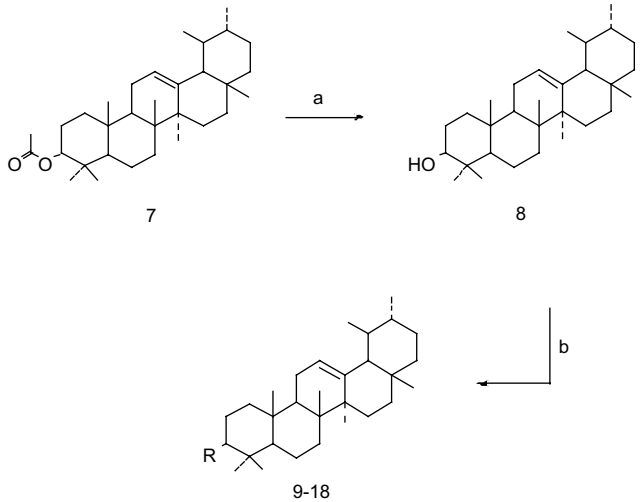
The syntheses of non-natural ester derivatives of α-amyrin acetate were accomplished by subjecting the naturally isolated α-amyrin actetate **7** to deacetylation by freshly prepared sodium methoxide in dry methanol. α-Amyrin **8** thus, obtained was esterified using various aromatic and aliphatic acids employing DCC–DMAP esterification protocol in dry DCM to afford non-natural esters of α-amyrin **9**–**18** (Scheme 1).

Excess of non-esterified fatty acids (NEFA) have been implicated in the pathology of insulin resistance [24]. High levels of NEFA have been linked in humans with insulin insensitivity [25]. Treatment with nicotinic acid decreases NEFA and increases peripheral glucose uptake, but this effect is not long lived [26]. Perhaps a more specific analogue of nicotinic acid, such as acipimox gave better results for controlling dyslipidemia and hyperglycemia [27]. Keeping this in view, we prepared few non-esterified aliphatic and aromatic acid derivatives of α-amyrin viz, **19**, **20** from the respective anhydrides in DMAP–DCM (Scheme 2).

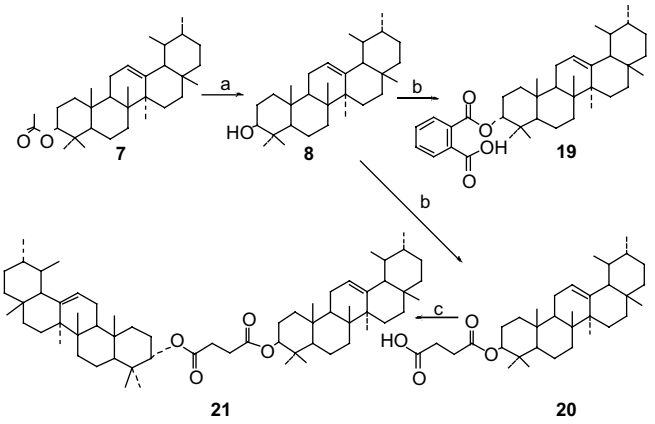
Since the active moiety was the amyrin scaffold itself, dimerization of the active factor was envisaged so as to enhance its antihyperglycemic activity. The synthesis was accomplished first by treating amyrin **8** with succinic anhydride and then reaction between the resultant succinyl derivative **20** and amyrin **8** to afford the target molecule **21** (Scheme 2).

Metformin **5** remains a primary therapeutic option for Type II diabetics [28]. New studies have described the effectiveness of combination therapy using metformin and other standard antihyperglycemics for the maintenance of blood glucose levels [29]. Keeping in view the efficacy of metformin in antidiabetic therapy, we envisaged Scheme 3 wherein thiourea unit resembling the metformin pharmacophore was incorporated in α-amyrin acetate in order to optimize the activity of the natural product lead.

The synthesis of the title compounds was accomplished by deacetylation of α-amyrin acetate **7** by sodium methoxide followed by oxidation of the resultant α-amyrin **8** to α-amyrinone **20** by PCC–DCM protocol. The usual methods available for hydrzone formation of saturated ketones failed in case of α-amyrinone. Hydrazine hydrate and its corresponding salt and phenyl hydrazine and its salt failed to convert the α-amyrinone **22** into the corresponding hydrazine derivative. So a strategy was devised in which a Lewis



Scheme 1. Reagents and conditions: (a) Sodium methoxide, methanol, r.t., 2 h. (b) Appropriate aromatic/aliphatic acid, DCC–DMAP, DCM, r.t., 4 h.



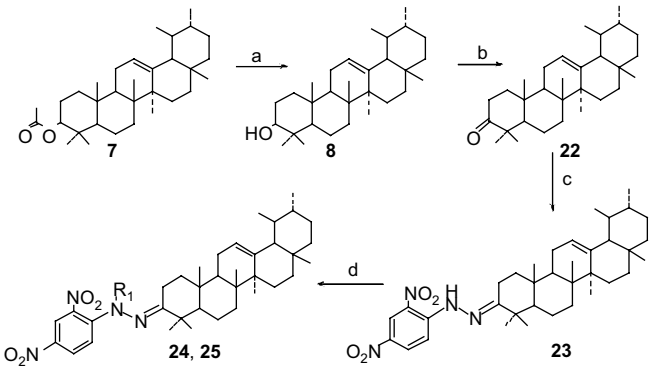
Scheme 2. Reagents and conditions: (a) Sodium methoxide, methanol, r.t., 2 h. (b) Appropriate anhydride, DMAP, DCM, r.t., 4 h. (c) α-Amyrin, DCC–DMAP, DCM, r.t., 6 h.

acid catalyst, indium chloride ($InCl_3$), hitherto unreported for hydrazine formation was employed for the same in order to facilitate the formation of the hydrazine **23** using more deactivating 2,4-dinitrophenylhydrazine.

The plausible mechanism was delineated to be of adduct formation of the catalyst with the sterically hindered ketone **22**. The corresponding hydrazine **23** was made to react with the appropriate phenylthioisocyanates in dry DMF in the presence of NaH resulting in the formation of corresponding thiourea derivatives **24** and **25** (Scheme 3).

2.2. Biology

All the compounds viz, the natural lead, α-amyrin acetate as well as its synthetic derivatives were evaluated for their antihyperglycemic activity in STZ-S model at the dose of 100 mg/kg selecting male albino rats of SD strain (body weight 140 ± 20 g). Fresh solution of streptozotocin solution (60 mg/kg) in 100 mM citrate buffer pH 4.5 and calculated amount of the fresh solution was injected to overnight fasted rats (45 mg/kg) intraperitoneally. Animals showing blood glucose values between 8 and 15 mM were selected and divided into groups of six animals in each. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at 100 mg/kg dose. A sucrose load of 2.5 g/kg body weight was given after 30 min of drug administration. After 30 min of post-sucrose load, blood glucose



Scheme 3. Reagents and conditions: (a) Sodium methoxide, methanol, r.t., 2 h. (b) Pyridinium chlorochromate, DCM, r.t., 12 h. (c) 2,4-Dinitrophenylhydrazine, absolute ethanol, indium chloride, reflux, 12 h. (d) Appropriate phenyl thioisocyanate, sodium hydride, DMF, 0 °C, r.t.

level was again checked with the aid of glucometer after 30, 60, 90, 120, 180, 240, 300 min and at 24 h, respectively. Comparison of the AUC of experimental and control groups determined the percent antihyperglycemic activity.

The activities were found to be very promising in few derivatives. α-Amyrin acetate **7** exhibited significant activity possessing antihyperglycemic activity of 18.4% ($P < 0.05$) and 17.0% ($P < 0.05$) at 5 and 24 h, respectively. Amongst the synthetic derivatives, the high potentiators of plasma glucose alleviation were **9**, **10**, **12**, **14** and **18** depicting respective lowering of 22.1% (5 h) and 25.6% (24 h; $P < 0.01$); 13.8% (5 h) and 27.6% (24 h; $P < 0.01$); 24.0% (5 h; $P < 0.01$) and 23.5% (24 h; $P < 0.01$); 18.2% (5 h) and 27.6% (24 h; $P < 0.01$); 24.9% (5 h; $P < 0.01$) and 20.2% (24 h; $P < 0.05$), respectively. The higher efficacy of these derivatives may be attributed to the presence of electron deactivating groups in **9**, **14** and **19** and lipophilic units in **12** and **18**. However, compound **15** bearing deactivating 2-quinonyl ring displayed diminished activity viz, 19.6% (5 h) and 17.8% (24 h; $P < 0.05$) than **14**, having 2-pyridyl ring. Moderate to good activity was also exhibited by **13**, the propenyl derivative with the percent glucose lowering of 12.7% (5 h; $P < 0.05$) and 22.6% (24 h; $P < 0.05$). The hydrophilic derivative **19** alleviated plasma glucose levels by 16.5% ($P < 0.05$) 24 h post-dose, although no significant lowering (11.0%) was observed at 5 h post-dose ($P < 0.05$). α-Amyrin **8** had a minimal effect on efficacy, 13.9% (5 h) and 8.12% (24 h; $P < 0.05$), substantiating the vitality of the acetate moiety in glucose amelioration effect. *p*-Methoxy phenyl acetic ester **11** reduced the glycemia by 17.5% (5 h; $P < 0.05$) and 15.6% (24 h; $P < 0.05$) revealing some insignificant role of the electron donating units in the hypoglycemia. The amino acid derivative **17** did not impart any prolific activity, 3.15% (5 h; $P < 0.05$) and 12.1% (24 h; $P < 0.05$) to the amyrin skeleton implying a lesser role for hydrophobicity in correcting hyperglycemia. In these experiments, the reference drug, metformin **5** lowered the blood glucose level by 23.5% ($P < 0.01$) at 5 h and 26.5% ($P < 0.01$) at 24 h at the same dose, respectively. Surprisingly, no marked activity was displayed by the thiourea derivative viz, **24** in the SLM model.

3. Conclusion

From the foregoing studies, it could be inferred that some of the non-natural ester analogues viz, **9**, **10**, **12**, **14** and **18** of the naturally occurring α-amyrin **10** are better glucose ameliorating than the naturally isolated α-amyrin acetate **7** and almost as equipotent as metformin. Moreover, it might be concluded from the above findings that lipophilicity and electron deficient moieties in the ester functionality are pivotal in the determination of plasma glucose alleviation. Further work is in progress to find out the mechanism of action in antihyperglycemic activity.

4. Experimental section

4.1. General

M.P. are uncorrected. IR spectra were recorded on a Perkin–Elmer RX-1 spectrophotometer using either KBr pellets or in neat. ^{1}H NMR and ^{13}C NMR, DEPT 90, DEPT 135 spectra were run on Bruker Advance DPX 200/300 MHz spectrometer in $CDCl_3$ unless stated otherwise. TMS was used as internal standard. FAB mass spectra were recorded on JEOL SX 102/DA-6000. Silica gel 60–120 mesh was used as stationary phase to isolate the compounds.

4.1.1. General procedure for the synthesis of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol (*8*)

To a stirred solution of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10, 11,12,12a,14,14a,14b-icosahydropicen-3-ylacetate **7** (4.68 g, 1 mmol) in dry methanol (7 ml) was added sodium methoxide (2.16 g, 4 mmol). The stirring was continued for 2 h at room temperature. The reaction mixture was acidified with amberlyst-120, filtered off under suction and the filtrate concentrated under reduced pressure to afford the desired compound. Yield: 92%; mp 180–181 °C; IR (KBr) 3349 (OH) and 1631 (C=C) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 3H), 0.83 (s, 3H), 0.87 (s, 6H), 0.93 (s, 3H), 0.96 (s, 3H), 0.99 (s, 3H), 1.13 (s, 3H), 2.17 (m, 2H), 3.22 (m, 1H), 5.12 (br s, 1H). FAB MS *m*/*z* 427 (M + 1).

4.1.2. General procedure for the synthesis of non-natural esters of amyrin (*9*–*19*)

To a stirred solution of 4,4,6a,8a,11,12,14b-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.85 g, 2 mmol) in dry DCM (8 ml) was added dicyclohexylcarbodiimide, DCC (0.82 g, 4 mmol), 4,4-*N*,*N*-dimethyl aminopyridine, DMAP (0.48 g, 4 mmol) and the appropriate acid (4 mmol). The stirring was continued for 4 h at room temperature. The reaction mixture was filtered off under suction and the filtrate concentrated under reduced pressure and the residue was subjected to silica gel chromatography using different polarity systems of hexane:ethylacetate.

4.1.3. General procedure for the synthesis of 4,4,8a,11,12,14b-hexamethyl-1,4,4a,5,6,6a,6b,7,8, 8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one (*22*)

To a stirred solution of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (1.04 g, 2.5 mmol) in dry DCM (15 ml) was added pyridinium chlorochromate, PCC (0.86 g, 4 mmol). The stirred was continued for 12 h at room temperature. The solvent was evaporated under reduced pressure and the residue was subjected to silica gel chromatography using hexane:ethylacetate (96:04) as the eluting solvent. Yield: 90%; mp 176–178 °C; IR (KBr) 1715 (CO) and 1631 (C=C) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 3H), 0.83 (s, 3H), 0.87 (s, 6H), 0.93 (s, 3H), 0.96 (s, 3H), 0.99 (s, 3H), 1.13 (s, 3H), 1.95 (m, 2H), 2.43 (m, 2H), 5.15 (br s, 1H). FAB MS *m*/*z* 425 (M + 1).

4.1.4. General procedure for the synthesis of (E)-1-(2,4-dinitrophenyl)-2-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexadecahydropicen-3-(4H,6bH,14bH)-ylidene)-hydrazine (*23*)

To a stirred solution of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexadeca hydropicen-3(4*H*,6b*H*,14b*H*)-one **22** (0.42 g, 1 mmol) in absolute ethanol (7 ml) was added 2,4-dinitrophenylhydrazine (0.198 g, 1 mmol) and indium chloride (0.022 g, 10 mol%). The reaction mixture was refluxed for 12 h. The solvent was evaporated under reduced pressure and the desired compound **23** was crystallized in methanol. Yield: 92%; mp 165–166 °C; IR (KBr) 3433 (NH) and 1631 (C=C) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 3H), 0.93 (s, 3H), 1.07 (s, 6H), 1.16 (s, 3H), 1.25 (s, 3H), 1.60 (s, 6H), 1.94 (m, 2H), 2.18 (m, 2H), 5.16 (t, $J = 6.7$ Hz, 1H), 7.95 (d, $J = 7.6$ Hz, 1H), 8.29 (dd, $J = 7.3$, 2.2 Hz, 1H), 9.12 (d, $J = 2.5$ Hz, 1H), 11.17 (br s, 1H). FAB MS *m*/*z* 605 (M + 1).

4.1.5. General procedure for the synthesis of thiourea derivatives of amyrin (*24*, *25*)

To a stirred solution of (*E*)-1-(2,4-dinitrophenyl)-2-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexa decahydropicen-3-(4*H*,6b*H*,14b*H*)-ylidene)-hydrazine **23** (0.60 g, 1 mmol) in dry DMF (6 ml) was added sodium hydride (0.23 g, 10 mmol) at 0 °C. The stirring was continued for 30 min at the same temperature. Then, the appropriate phenyl thioisocyanate (4 mmol) was added to the ice-cold mixture and stirring continued for overnight at room temperature. The reaction mixture was plunged into ice-cold water, filtered under suction and filtrate extracted with

chloroform (4 × 25 ml). The organic layer was dried upon sodium sulphate, concentrated under reduced pressure and the residue was subjected to silica gel chromatography, eluting with hexane:ethylacetate (90:10) solvent system.

4.2. Synthesis of derivatives

4.2.1. Synthesis of 4,4,6a,6b,8a,11,12,14b-octa methyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-4-chloro benzoate (**9**)

To a stirred solution of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8**(0.85 g, 2 mmol) in dry DCM (10 ml) was added DCC (0.82 g, 4 mmol), DMAP (0.48 g, 4 mmol) and 4-chlorobenzoic acid (0.56 g, 4 mmol). The stirring was continued for 4 h at room temperature. The reaction was filtered off under suction, concentrated under reduced pressure and the concentrate was subjected to silica gel chromatography using hexane:ethylacetate (94:06) system as eluent. Yield: 80%; mp 161–163 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 6H), 0.93 (s, 6H), 1.02 (s, 6H), 1.15 (s, 3H), 1.25 (s, 3H), 1.91 (m, 2H), 1.95 (m, 2H), 4.73 (t, J = 5.7 Hz, 1H), 5.13 (br s, 1H), 7.41 (d, J = 8.3 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H). FAB MS m/z 565 (M + 1).

4.2.2. Synthesis of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-4-nitrobenzoate (**10**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.85 g, 2 mmol) and 4-nitrobenzoic acid (0.66 g, 4 mmol) by DCC–DMAP in dry DCM. Yield: 72%; mp 192–193 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 6H), 0.93 (s 6H), 1.03 (s, 6H), 1.15 (s, 3H), 1.25 (s, 3H), 1.91 (m, 2H), 1.93 (m, 2H), 4.73 (t, J = 5.7 Hz, 1H), 5.14 (br s, 1H), 8.19 (d, J = 8.8 Hz, 2H), 8.29 (d, J = 8.7 Hz, 2H). FAB MS m/z 576 (M + 1).

4.2.3. Synthesis of 4,4,6a,6b,8a,11,12,14b-octa-methyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-2-(4-methoxy ph enyl)acetate (**11**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.42 g, 1 mmol) and 4-methoxyphenyl acetic acid (0.33 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 54%; mp 148–150 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.76 (s, 6H), 0.79 (s, 3H), 0.80 (s, 3H), 0.90 (s, 3H), 0.95 (s, 3H), 0.99 (s, 3H), 1.05 (s, 3H), 1.56–1.64 (m, 2H), 1.86–1.92 (m, 2H), 3.54 (s, 2H), 3.78 (s, 3H), 4.48 (t, J = 5.8 Hz, 1H), 5.11 (t, J = 6.8 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H). FAB MS m/z 575 (M + 1).

4.2.4. Synthesis of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-5-oxo-5-phenyl pentanoate (**12**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.42 g, 1 mmol) and 4-benzoyl butyric acid (0.38 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 72%; mp 187–190 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.79 (s, 6H), 0.86 (s, 6H), 0.91 (s, 3H), 0.97 (s, 3H), 1.00 (s 3H), 1.06 (s, 3H), 1.91 (m, 2H), 1.88–1.93 (m, 2H), 2.10 (m, 2H), 2.42 (t, J = 7.0 Hz, 2H), 3.05 (t, J = 7.1 Hz, 2H), 4.53 (t, J = 7.7 Hz, 1H), 5.12 (br s, 1H), 7.42–7.56 (m, 3H), 7.96 (d, J = 7.1 Hz, 2H). FAB MS m/z 601 (M + 1).

4.2.5. Synthesis of (E)-4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-2-methylbut-2-enoate (**13**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.43 g, 1 mmol) and 2-methyl butanoic acid (0.20 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 46%; mp 160–162 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.72 (s, 6H), 0.81 (s, 3H), 0.84 (s, 6H), 0.92 (s, 3H), 0.94 (s, 3H), 1.00 (s, 3H), 1.44 (m, 2H), 1.58 (s, 3H), 1.59 (s, 3H), 1.69–1.73 (m, 2H), 1.76–1.82 (m, 2H), 4.43–4.50 (m, 1H), 5.05 (t, J = 6.7 Hz, 1H), 6.76 (q, J = 6.9 Hz, 1H). FAB MS m/z 509 (M + 1).

4.2.6. Synthesis of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-nicotinate (**14**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **10** (0.42 g, 1 mmol) and pyridine-2-carboxylic acid (0.24 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 53%; mp 220–221 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 6H), 0.93 (s, 6H), 1.09 (s, 6H), 1.15 (s, 3H), 1.25 (s, 3H), 1.93 (m, 2H), 1.96 (m, 2H), 4.78 (t, J = 8.1 Hz, 1H), 5.14 (br s, 1H), 7.36–7.42 (m, 1H), 8.29 (d, J = 7.9 Hz, 1H), 8.76–8.78 (m, 1H), 9.23 (br s, 1H). FAB MS m/z 532 (M + 1).

4.2.7. Synthesis of 4,4,6a,6b,8a,11,12,14b-octa-methyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-isoquinoline-1-carboxylate (**15**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.42 g, 1 mmol) and quinoline-2-carboxylic acid (0.37 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 51%; mp 205–206 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR δ 0.81 (s, 3H), 0.92 (s, 3H), 1.00 (s, 6H), 1.04 (s, 6H,), 1.09 (s, 6H), 1.81 (m, 2H), 1.92–1.96 (m, 2H), 4.92 (t, J = 8.2 Hz, 1H), 5.14 (br s, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 8.29 (1H, d, J = 8.2 Hz, H-7′), 8.33 (1H, d, J = 8.2 Hz, H-4′). FAB MS m/z 582 (M + 1).

4.2.8. Synthesis of 4,4,6a,6b,8a,11,12,14b-octa-methyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-2-bromopropanoate (**16**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.43 g, 1 mmol) and 2-bromopropanioc acid (0.30 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 35%; mp 196–199 °C; 562 (M + 2); IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.79 (s, 6H), 0.90 (s, 9H), 0.99 (s, 3H), 1.01 (s, 3H), 1.07 (s, 3H), 1.25 (s, 3H), 1.66–1.68 (m, 2H), 1.80–1.85 (m, 2H), 4.36 (q, J = 7.5 Hz, 1H), 4.52–4.59 (m, 1H), 5.13 (t, J = 3.5 Hz, 1H). FAB MS m/z 561 (M + 1).

4.2.9. Synthesis of 4,4,6a,6b,8a,11,12,14b-octa-methyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-2-benzamido acetate (**17**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.42 g, 1 mmol) and *N*-benzoylglycine (0.35 g, 2 mmol) by DCC–DMAP in dry DCM. Yield: 43%; mp 94–96 °C; IR (KBr) 1735 (CO), 1689 (CONH) and 1631 (C═C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$)

δ 0.80 (s, 3H), 0.82 (s, 3H), 0.85 (s, 3H), 0.98 (s, 3H), 1.01 (s, 3H), 1.07 (s 3H), 1.09 (s, 3H), 1.25 (s, 3H), 1.90 (m, 2H), 1.95 (m, 2H), 4.24 (d, $J = 4.9$ Hz, 2H), 4.62 (t, $J = 7.8$ Hz, 1H), 5.12 (t, $J = 3.3$ Hz, 1H), 6.72 (br s, 1H), 7.44–7.52 (m, 3H), 7.81 (dd, $J = 8.1$, 1.2 Hz, 2H). FAB MS m/z 588 (M + 1).

4.2.10. Synthesis of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-2,5-dimethyl benzoate (**18**)

By the analogous procedure as described for compound **9**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4, 4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.43 g, 1 mmol) and 2,5-dimethylcarboxylic acid (0.30 g, 2 mmol) by DCC–DMAP in dry DCM. The stirring was continued for 4 h at room temperature. The reaction was filtered off under suction, concentrated under reduced pressure and the desired compound was isolated by silica gel chromatography. Yield: 43%; mp 202–203 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 3H), 0.91 (s, 3H), 0.96 (s, 6H), 0.99 (s, 3H), 1.02 (s, 3H), 1.08 (s 3H), 1.25 (s, 3H), 1.81 (m, 2H), 1.92–1.96 (m, 2H), 2.34 (s, 3H), 2.55 (s, 3H), 4.75 (t, $J = 8.1$ Hz, 1H), 5.12 (br s, 1H), 7.09–7.21 (m, 2H), 7.69 (s, 1H). FAB MS m/z 558 $(M)^+$.

4.2.11. Synthesis of 2-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yloxy)-carbonyl-benzoic acid (**19**)

To a stirred solution of 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.42 g, 1 mmol) in dry DCM (10 ml) was added DMAP (0.48 g, 4 mmol) and phthalic anhydride (0.59 g, 4 mmol). Yield: 42%; mp 193–194 °C; IR (KBr) 1735 (CO), 1740 (COOH) and 1631 (C═C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 6H), 0.91 (s, 6H), 0.98 (s, 6H), 1.01 (s, 3H), 1.08 (s, 3H), 1.89 (t, $J = 11.3$ Hz, 2H), 4.77 (m, 1H), 5.13 (br s, 1H), 7.58 (d, $J = 7.3$ Hz, 2H), 7.73 (d, $J = 4.7$ Hz, 1H), 7.90 (d, $J = 4.6$ Hz, 1H). FAB MS m/z 575 (M + 1).

4.2.12. Synthesis of 4-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yloxy)-4-oxo-butanoic acid (**20**)

By the analogous procedure as described for compound **19**, was obtained from 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a, 6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.85 g, 2 mmol) and succinic anhydride (0.20 g, 2 mmol) by DMAP in dry DCM. Yield: 48%; mp 170–175 °C; IR (KBr) 1735 (CO), 1740 (COOH) and 1631 (C═C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$,) δ 0.79 (s, 6H), 0.86 (s, 6H), 0.91 (s, 3H), 0.97 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H), 1.91 (t, $J = 8.5$ Hz, 2H), 2.66 (m, 4H), 4.54 (m, 1H), 5.12 (br s, 1H). FAB MS m/z 527 (M + 1).

4.2.13. Synthesis of 4,4,6a,8a,11,12,14b-hepta-methyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-yl-succinate (**21**)

By the analogous procedure as described for compound **9**, was obtained from 4-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5, 6,6a,6b,7,8,8a,9,10,11,12,12, 14,14a,14b-icosahydropicen-3-yloxy)-4-oxobutanoic acid **20** (0.26 g, 0.5 mmol) and 4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol **8** (0.21 g, 0.5 mmol) by DCC–DMAP in dry DCM. Yield: 52%; mp >210 °C; IR (KBr) 1735 (CO) and 1631 (C═C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.79 (s, 12H,), 0.86 (s, 12H), 0.91 (s, 6H), 0.97 (s, 6H), 1.00 (s, 6H), 1.06 (s, 6H), 1.91 (t, $J = 8.2$ Hz, 4H), 2.63 (m, 4H), 4.53 (m, 2H), 5.12 (br s, 2H). FAB MS m/z 921 (M + 1).

4.2.14. Synthesis of (E)-2-(2,4-dinitrophenyl)-1-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexadecahydropicen-3(4*H*,6b*H*,14b*H*)-ylidene)-4-phenylthiosemicarbazide (**24**)

By the analogous procedure as described in the representative method for thiourea derivatives, was obtained from (*E*)-1-(2,4-dinitrophenyl)-2-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexadecahydropicen-3(4*H*,6b*H*,14b*H*)-ylidene) hydrazine **23** (0.60 g, 1 mmol) and phenyl thioisocyanate (0.54 g, 4 mmol) by NaH in dry DMF. Yield: 18%; mp 152–153 °C; IR (KBr) 3421 (NH), 1624 (C═C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.77 (s, 6H), 0.79 (s, 6H), 1.03 (s, 9H), 1.10 (s, 3H), 1.90 (t, $J = 8.2$ Hz, 2H), 5.10 (br s, 1H), 6.71 (d, $J = 8.9$ Hz, 1H), 7.42–7.49 (m, 3H), 7.55 (dd, $J = 7.4$, 1.3 Hz, 2H), 8.00 (dd, $J = 8.9$, 2.2 Hz, 1H), 8.25 (d, $J = 2.1$ Hz, 1H). FAB MS m/z 740 (M + 1).

4.2.15. Synthesis of (E)-2-(2,4-dinitrophenyl)-4-(2-nitrophenyl)-1-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexadecahydropicen-3-(4H,6bH,14bH)-ylidene)-thiosemicarbazide (**25**)

By the analogous procedure as described for compound **24**, was obtained from (*E*)-1-(2,4-dinitrophenyl)-2-(4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a-hexadecahydropicen-3-(4*H*,6b*H*,14b*H*)-ylidene)-hydrazine **23** (0.45 g, 0.75 mmol) and 2-nitrophenylthioisocyanate (0.72 g, 4 mmol) by NaH in dry DMF. Yield: 12%; semisolid; IR (KBr) 3433 (NH), 1631 (C═C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.79 (s, 6H), 0.82 (s, 3H), 0.85 (s, 6H), 1.25 (s, 9H), 2.14 (t, $J = 6.7$ Hz, 2H), 5.10 (br s, 1H), 6.46 (d, $J = 8.9$ Hz, 1H), 7.36–7.43 (m, 3H), 7.53 (d, $J = 8.8$ Hz, 1H), 8.02 (dd, $J = 8.7$, 2.2 Hz, 1H), 8.28 (d, $J = 2.1$ Hz, 1H). FAB MS m/z 785 (M + 1).

4.3. Antihyperglycemic assay

4.3.1. Sucrose-loaded (SLM) model

Overnight fast male albino rats were used for sucrose-loaded experiment. Blood was collected initially and thereafter test compounds were given to the test group consisting of five rats by oral gavage at a dose of 100 mg/kg body weight. After half an hour post-test treatment, a sucrose load of 10 mg/kg body weight was given to each rat. Blood was collected at 30, 60, 90 and 120 min post-sucrose load. The % fall in blood glucose level was calculated according to the AUC method.

4.3.2. Streptozotocin (STZ-S) model

Male albino rats of SD strain (body weight 140 ± 20 g) were selected for this study. Streptozotocin was dissolved in 100 mM citrate buffer pH 4.5 and calculated amount of the fresh solution was injected to overnight fasted rats (45 mg/kg) intraperitoneally. Blood was checked 48 h later by glucostrips and animals showing blood glucose values between 8 and 15 mM were selected and divided into groups of six animals in each. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at 100 mg/kg dose. Animals of control group were given an equal amount of 1.0% gum acacia. A sucrose load of 2.5 g/kg body weight was given after 30 min of drug administration. After 30 min of post-sucrose load, blood glucose level was again checked at 30, 60, 90, 120, 180, 240, 300 min and at 24 h, respectively. Comparison of the AUC of experimental and control groups determined the percent antihyperglycemic activity.

Acknowledgements

Authors are grateful to the Director, CDRI, Lucknow, India for constant encouragement for the program on antihyperglycemic

drugs. We also thank the RSIC for spectral data and K.K. Joshi for plant collection. Tanvir is thankful to ICAR and ICMR, New Delhi for financial support.

Appendix. Supplementary material

Supplementary material associated with this article can be found in the online version, at doi: 10.1016/j.ejmech.2008.09.011.

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