Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2763–2772

BIOORGANIC & MEDICINAL CHEMISTRY

Modified Iridoid Glycosides as Anti-implantation Agents: Inhibition of Cell Adhesion as an Approach for Developing Pregnancy Interceptive Agents☆

Anju P. Misra,[a] Vijayavitthal T. Mathad,[a] Kanwal Raj,[a,*] Amiya P. Bhaduri,[a] Rashmi Tiwari,[b] Anuradha Srivastava[b] and P.K. Mehrotra[b]

[a]*Medicinal Chemistry Division, Central Drug Research Institute, Lucknow 226 001, India*
[b]*Endocrinology Division, Central Drug Research Institute, Lucknow 226 001, India*

Received 10 December 1999; accepted 10 March 2001

Abstract—Structural modifications in iridoid glycosides and evaluation of their efficacy on adhering capability (in vitro) of immature hamster uterine epithelial cells to the substratum have been studied. Out of 31, eight compounds in vitro, five compounds in utero and two in vivo showed adhesion/implantation preventing activity, respectively. The results provide an indication for further exploration in the line of development of anti-adhesive agents. © 2001 Published by Elsevier Science Ltd.

Introduction

Iridoid glycosides, a class of natural product, are associated with a wide variety of biological activities.[1–10] A programme of research was drawn to carry out structural modifications in iridoid glycoside for evoking cell-specific responses. In this connection, three distinct areas of research activities were identified for the biological evaluations of structurally modified iridoid glycosides. The first one was concerned with immune responses while the second one was concerned with activation of macrophages responsible for wound healing and the third one was aimed at preventing adhesion of the blastocyst to the uterine epithelial cell surface, an approach to counter unwanted pregnancies. The immuno-modulatory effect of modified iridoid glycosides has been published earlier[11] and the wound healing activity will be reported elsewhere. The present communication is concerned with providing experimental evidences for the projected objective.

Implantation of embryo to the uterine epithelium requires sensitisation of both the tissues for preparation of successful pregnancy. Hence, synchronisation of the two tissues are required for switching over to receptive stage from a functionally non-receptive stage.[12a] During the receptive stage, a marked reduction in the expression of MUCINE (MUC-1)[12b–d] at the luminal surface of the uterine epithelial cell is reported in mouse, rat and rabbit.[13–15] As a result of this reduced expression, the epithelial cells become receptive for the adherence of trophoblast,[16] the cells that encapsulate implanting the blastocyst. Since MUC-1 is part of a complex extra cellular matrix of the epithelial cell, it may be argued that chemical entities capable of interacting with the extra cellular matrix of the epithelial cells could lead to rejection of the blastocyst destined for implantation. This approach, therefore, requires the development of tissue specific cell–cell adhesion blockers and in order to meet this requirement, modified iridoid glycosides have been synthesised and their efficacies as anti-implantation agents have been explored. The details are presented here.

Chemistry

Methyl ester of the iridoid glycoside, loganin (**1**), was hydrolysed to the corresponding acid (**2**)[17,18] and the resulting loganic acid was acetylated to loganic acid pentaacetate (**3**). The active ester of (**3**) was prepared by coupling it with 1-hydroxybenztriazole (HOBT) in the presence of 1,3-dicyclohexylcarbodiimide (DCC). Reactions of the active ester (**4**) with different amines and amino acid methyl ester furnished the corresponding amides (**5**–**17**). Deacetylation of these amides was

☆CDRI Communication No. 5975.

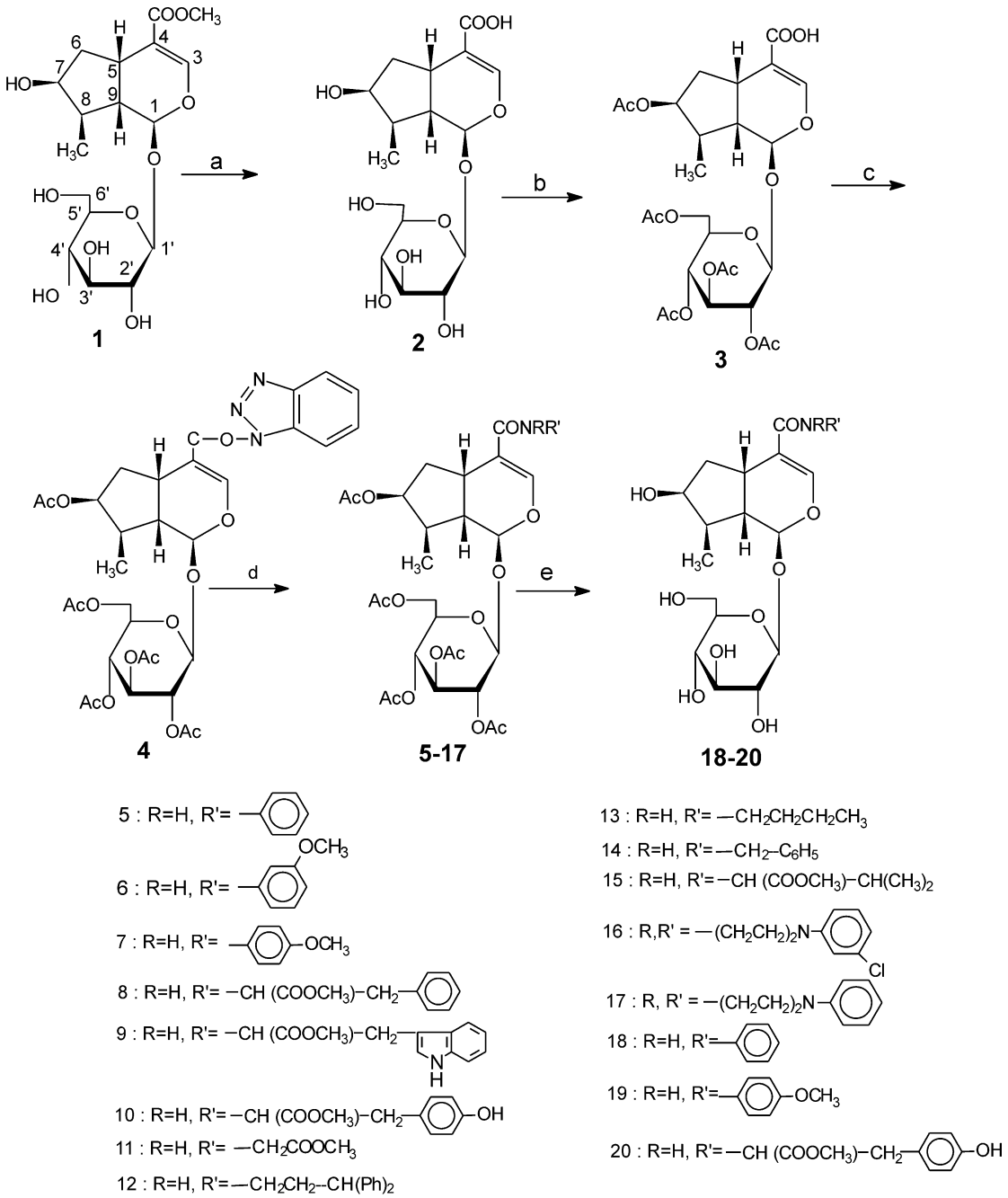
*Corresponding author. Tel.: +91-212411-18, ext. 4338; fax: +91-522-2234505/223938; e-mail: root@cscdri.ren.nic.in

0968-0896/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0968-0896(01)00105-5

carried out by sodium methoxide in methanol (catalytic amount of sodium in dry methanol) to yield the hydroxy derivatives **18**–**20** (Scheme 1). In another strategy, the direct synthesis of amides from loganic acid (**2**) was accomplished by coupling it with either EDCI [1-ethyl-3-(3′-dimethylamino) propyl carbodiimide] or with BOP [benzotriazol-1-yloxy) tris (dimethyl amino) phosphonium hexafluoro phosphate] and was followed by reacting the active ester with the desired amines. Compounds **18**–**20** were prepared by this method (Scheme 2). The aglucone (**21**) of loganin (**1**) was prepared by reacting it with β-glycosidase.[19] Reaction of aglucone with different alcohols in the presence of boron-trifluoride-etherate yielded α/β-isomeric mixture of ethers (**22**–**26**).[19] Hydrolysis of compound **22** with sodium hydroxide yielded the corresponding acid (**27**), which was coupled with HOBT to deliver **28**, which in turn furnished amides **29**–**30** on reaction with different amines (Scheme 3). 6-Tosyl α-glucopyranoside, on reaction with sodium hydroxide in methanol, gave **32,** which was then reacted with cinnamic anhydride to furnish the disubstituted derivative **33** (Scheme 4).

Results and Discussion

Thirty-one synthesised compounds, **1**–**30** and **33**, were evaluated for anti-adhesive activity in cell–cell adhesion



Scheme 1. Reagents: (a) $Ba(OH)_2/CH_3OH$; H^+; (b) Ac_2O/pyridine; (c) DCC, HOBT, CH_2Cl_2; (d) amines/CH_2Cl_2; (e) Na, CH_3OH.

(in vitro) at different molar concentrations. Of these, two compounds **7** and **9**, caused degeneration at the maximum dose level, that is (80×10^{-7}) M concentration. Four compounds, **4**–**6** and **26**, prevented the attachment of the cell seeded on multi-well plate at (40×10^{-7}) M concentrations, whereas compounds **8** and **10** caused complete degeneration at 20×10^{-7} M concentration (Table 1). Other compounds showed no effect at maximum dose.

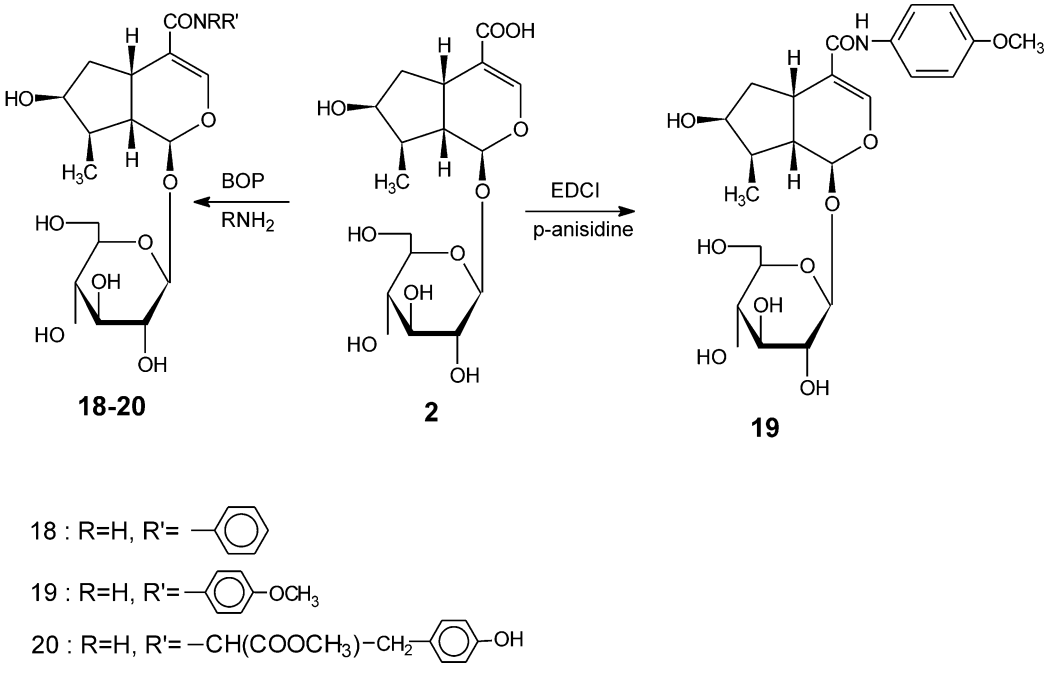
The compounds that demonstrated in-vitro activity were subjected to in-utero study. Compounds **4**, **7** and **10** caused foetal resorption at higher dose (20 μL) and compared to these administration of compound **6** resulted in failure of implantation at a lower dose (10 μL). Compound **8**, on the other hand, not only arrested the implantation of blastocyst but also changed the appearance of the uterus at 10 μL. This was evident by the sign of complete resorption in treated horn. The control horn, however, had normal implantation (Table 2). The other three compounds, **5**, **9** and **26** (not reported), were found inactive.

Anti-implantation activity (in vivo) was evaluated only when the compounds were found active in in-vitro and in-utero test systems. Among them, only compound **8** exhibited centpercent implantation preventing efficacy while compound **7** showed 50% efficacy. Compounds **4**, **6** and **10** were, however, found ineffective in arresting implantation (Table 3).

The present study was aimed at identifying one modified iridoid glycoside which would exhibit efficacy in preventing cell adhesion in vitro, implantation of embryo on administration in utero and implantation after subcutaneous administration in vivo. Compound **8** was thus identified as the chemical entity which, by preventing cell adhesion, might have prevented the implantation of the blastocyst. It is difficult to say that the process of implantation was inhibited by the compound directly or by evoking permeability changes in the cell membrane, which in turn would have affected the receptive surface of the epithelial cell. The chemical structure of compound **8** suggests that the oestrogenic and anti-oestrogenic activity may not be associated with this compound. It is therefore logical to conclude that the compound might not have acted as a hormone response modifier. It can then be concluded that although the exact mechanism by which this compound exhibit anti-implantation activity is not yet clear, the circumstancial evidence suggests that the extracellular matrix of the uterine cell May have been disturbed enough by not permitting molecular recognition of the trophoblast extracellular matrix, leading finally to the failure of adhesion. The results presented here, therefore, provoke further studies in this area and are likely to provide a completely new approach in preventing unwanted pregnancies.

Anti-adhesive activity evaluation

Attachment of free-floating blastocyst to the apical surface of the uterine epithelial cells involves the process of the cell–cell adhesion. The experimental demonstration of this process is either to co-culture the two cell types or to culture them separately on the surface of culture dishes. Chemical entities assumed to exhibit an anti-adhesive property could then be tested on the uterine epithelial cells by monitoring their ability to prevent attachment of cells to the substratum of culture dishes. The compounds which prevented the attachment of cells when co-incubated with the compounds were later tested in utero (by injecting the compounds intraluminally in the uterus prior to the adhesion of embryo) and then in vivo (by administering them to pregnant animals in the treatment schedule that



Scheme 2.

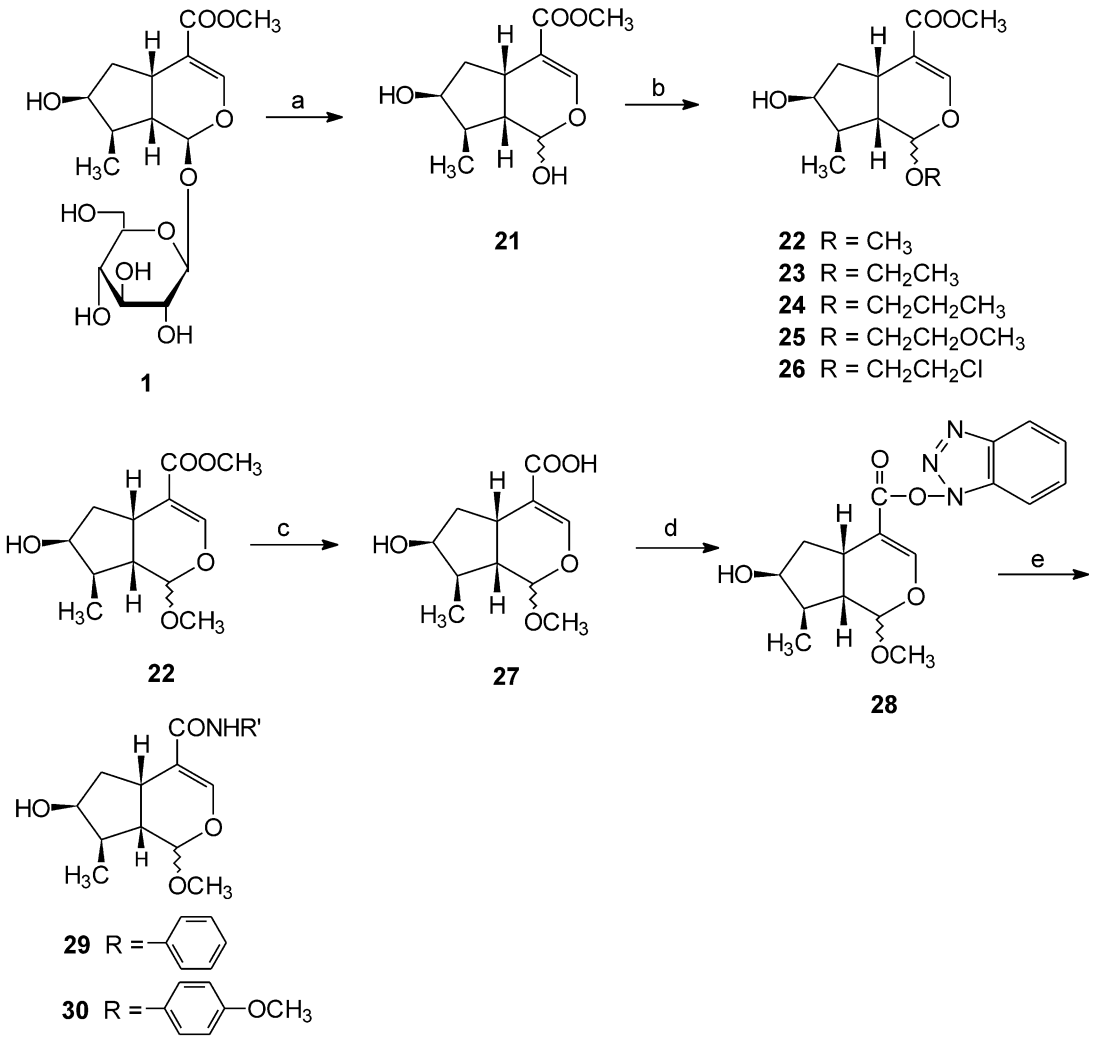
covers pre- and peri-attachment stages of implantations).

In-vitro. Isolation of uterine epithelial cells. Premature female (30–40 g) syrian hamsters (*Mesoricetus auratus*), bred in the Institute facility were used. They were primed with estradiol dipropionate 0.1 μg/animals) and progesterone (1.0 mg/animal) 24 h before autopsy. By priming the animals with these hormones, recovery of the cells became very high.

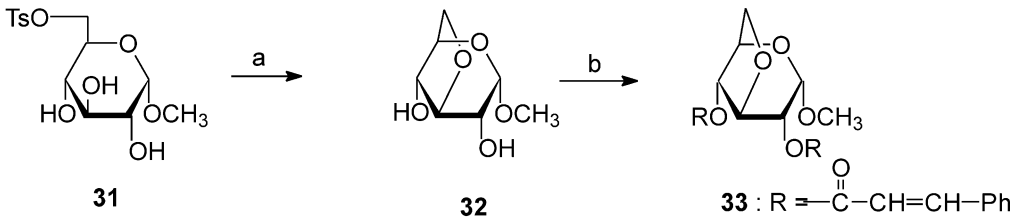
The animals were autopsied following inhalation of anaesthetic ether (Hydroquinone). Laparotomy was done to dissect out the uterine horns which, after cleaning off mesenchyme and other adhered tissues, were placed in phosphate buffer saline. Each horn was cut into small pieces (1.0 mm^3). These pieces were washed with buffer (×3) prior to further processing through enzyme mixture for isolation of epithelial cells. The cells were isolated by exposing the minced tissue to trypsin, pancreatin and DNase mixture, in which they were digested on a Dubnoff metabolic shaker at (4 °C) for 30 min. Thereafter, the buffer was aspirated (in and out) with sterile dropper several times to enable the cells to become loose. The aliquot containing suspended cells was washed initially with buffer and then with Dulbecco's minimum essential medium (DMEM) prior to centrifugation at 1000*g* for 2 min.

The resultant pallet was washed with medium (three times). Finally the cells were suspended in complemented (10% foetal caff serum) medium. A desired quantity (10–20 μL) of cell suspension was placed in the wells of a 96-well cultured plastic plate (NUNC, Denmark) containing complemented medium (100 μL). Prior to seeding, the cells were checked by the Trypan Blue dye exclusion method for viability and seeded only if the number exceeded 90% of the viable cells. All the biochemical were procured from Sigma Chemical Company, St. Louis, USA.

Preparation of the solution of test compounds. The compounds designed for testing anti-adhesive properties, were initially dissolved in DMSO and then subsequently diluted with the medium in different molar concentrations and were injected at '0' hour. The working solution contained very neglible amount (less than 0.1% v/v) of DMSO. However, in order to check if the DMSO has any adverse effect on the growth of



Scheme 3. Reagents: (a) β-glucosidase/citrate buffer, pH 5; (b) $ROH/BF_3{\cdot}Et_2O$; (c) $NaOH/H^+$; (d) $DCC\text{-}HOBT/CH_2Cl_2$; (e) $amines/CH_2Cl_2$.



31 32

Scheme 4. Reagents: (a) $NaOH/CH_3OH$; (b) $(RCO)_2O$/pyridine.

Table 1. In-vitro evaluation of compounds designed for anti-adhesive activity on uterine epithelial cells of immature hamster

Compound no.	Concentration (10^{-7} molar) (μL)	Observation[a] (24 h)	Observation[a] (48 h)	Comments
Vehicle	10	+ + + +	+ + + +	No effect
4	10	+ + + +	+ + + +	No effect
	20	+ + +	+ +	Partial effect—48 h
	40	+ +	–	Degeneration—48 h
	80	–	–	Degeneration—24 h
5	10	+ + + +	+ + + +	No effect
	20	+ + +	+ +	Partial effect—48 h
	40	+ +	–	Degeneration—48 h
	80	–	–	Degeneration—24 h
6	10	+ + + +	+ + + +	No effect
	20	+ + +	+ +	Partial effect—48 h
	40	+ +	–	Degeneration—48 h
	80	–	–	Degeneration—24 h
7	10	+ + + +	+ + + +	No effect
	20	+ + + +	+ + + +	-do-
	40	+ + +	+ + +	mild effect 24 h
	80	+ +	–	Degeneration—48 h
8	10	+ + + +	+ + + +	No effect
	20	+ +	–	Degeneration—48 h
	40	–	–	Degeneration—24 h
9	10	+ + + +	+ + + +	No effect
	20	+ + + +	+ + + +	No effect
	40	+ + +	+ + +	mild effect
	80	+ +	–	Degeneration—48 h
10	10	+ + + +	+ + + +	No effect
	20	+ +	–	Degeneration—48 h
	40	–	–	Degeneration—24 h
26	10	+ + + +	+ + + +	No effect
	20	+ + +	+ +	Partial effect—48 h
	40	+ +	–	Degeneration—48 h
	80	–	–	Degeneration—24 h

[a](+) and (–) signs represent presence/absence of attachment and growth or degeneration; 1–4 (+) indicate the percentage of cells (25–100, respectively).

these cells a greator quantity of it (1.0–10.0 μL) was tested.

Examination of the culture. The culture was examined over the inverted microscope 24 and 48 h after seeding of the cell (0 h). Attachment, growth, proliferation or monolayer formation were considered as parameters for healthy cells. Arrest of attachment (24 h) or further growth (48 h) was considered as the potentiality marker of the compound.

In utero. Adult female hamsters (70–80 g) were mated with coeval males overnight in 3:1 ratio and the vaginal smear was examined following morning which was considered as day 1 post coitum (pc). On day 3 pc the sperm positive females were laparotomized (under controlled ether anaesthesia) by making minor incision on the lower side of the abdomen. Through this, the uterine horns were taken out by medium sized forceps. The test compounds dissolved in 50% DMSO, were injected in micro quantity (5, 10 and 20 μL) of molar concentration in one horn and the vehicle (DMSO) in the same quantity in the other horn. To prevent spillage, the uterine horn were tied by a black thread at the utereo-vaginal junction and then placed back in the peritoneum. The abdomen was stitched with thread. Acriflavin antiseptic was applied at the operation site and the animals were injected with Terramycin (Pfizer, India) for 3 days for speedy healing of the injury.

The treated animals were again laparotomised on day 8 of pregnancy to monitor the effect of compounds on implantation and, if it had occurred, the subsequent development of foetuses in the treated horn. Corpora lutea (a differentiated stage of ovarian follicle representing ovulation points) were also counted simultaneously. The data was recorded and analysed for depicting the efficacy of the compounds.

In vivo. Mated female hamsters were taken and on day 3–5 of pregnancy the compounds (by dissolving in 50% DMSO) were administered by subcutaneous (sc) route. The control animals received vehicle (50% DMSO) only. The treated animals were laprotomised on day 10 of pregnancy and the number of implantations (healthy or otherwise) or resorption sites (indicative of arrest in growth of developing embryos) were counted. Corpora lutea of the respective sides were also counted. The efficacy of the compound was analysed by deducting the number of animals showing absence of implantations and/or resorption from those showing normal implantation and/or developing foetuses.

Experimental

Melting points were recorded on a hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker 400 FT NMR, Bruker Avance DRX 300 and Bruker DPX 200 spectrometers, using TMS as internal reference. FAB mass spectra were recorded on JEOL SX 102/DA 600 mass spectrometer using Argon/xenon (6KV, 10 mA) as the FAB gas, EI mass spectra on JEOL JMS-D-300 spectrometer with the ionization potential of 70 eV and ES mass on Quatro-II, micro mass. Elemental analysis were carried out on a Carlo-Erba EA 1108 analyser. ^{1}H NMR of the loganic acid pentaacetate was assigned on the basis of HOMOCOSY

experiment and rest of the compounds were compared with it.

HOBT ester of loganic acid pentaacetate (4)

To a solution of loganic acid penta acetate (1.0 gm, 1.7 m mol) in dry dichloro methane (30 mL) was added DCC (351 mg, 1.7 mmol), followed by HOBT (229 mg, 1.7 mmol) at room temperature and stirring was continued until the clear solution turned into suspension. Separated DCU was filtered off and the filtrate was concentrated to a white foamy mass and purified by column chromatography. Elution with ethylacetate/hexane (15:85) gave the desired compound 770 mg.

Yield: 63%; mp 65 °C; IR (KBr): 1752 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 8.07 (d, 1H, $J=9$ Hz, Ar–H), 7.78 (s, 1H, H-3), 7.56–7.41 (m, 3H, Ar–H), 5.36 (d, 1H, $J=3$ Hz, H-1), 5.27–4.91 (m, 5H, H-1′-H-4′ and H-7), 4.33 (dd, 1H, $J=12$, 4 Hz, H-6′), 4.20 (dd, 1H, $J=12$, 2 Hz, H-6′), 3.79 (m, 1H, H-5′), 3.24 (m, 1H, H-5), 2.37 (m, 1H, H-6), 2.32 (m, 1H, H-9), 2.11, 2.06, 2.05, 2.03, 1.95 (s, 3H each, $-COCH_3$), 1.95 (m, 1H, H-8), 1.93 (m, 1H, H-6), 1.10 (d, 3H, $J=6.8$ Hz, H-10). MS (FAB): m/z 704 ($M^+ + 1$). Anal. calcd for $C_{32}H_{37}N_3O_{15}$: C, 54.62; H, 5.26; N, 5.97. Found: C, 54.80; H, 5.26; N, 6.14%; [α] −72.30 (c 0.26 in methanol).

Amidation of loganic acid pentaacetate: a general procedure (5–17)

To a solution of HOBT ester of loganic acid pentaacetate **4** (1.0 gm. 1.39 mmol) in dry dichloromethane (25 mL) aniline was added (0.129 mL, 1.39 mmol) and stirred for 4 h. The solid that separated was filtered off. Filtrate was concentrated to a thick mass which was purified by column chromatography. On elution with ethyl acetate/hexane (20:80) gave the desired compound **5**, 770 mg.

Compound 5. Yield: 83%; mp 167 °C; IR (KBr): 1754 cm^{-1} (>=O), 1634 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$); δ 7.85–7.10 (m, 6H, H-3, Ar–H), 5.29–4.89 (m, 6H, H-1′, H-4′, H′1 and H-7), 4.33 (dd, 1H, $J=12$, 4 Hz, H-6′), 4.14 (dd, 1H, $J=12$, 2 Hz, H-6′), 3.79 (m, 1H, H-5′), 3.12 (m, 1H, H-5), 2.36 (m, 1H, H-6), 2.30 (m, 1H, H-9), 2.10, 2.04, 2.03, 1.99, 1.93 (s, 3H each, $-COCH_3$), 1.93 (m, 1H, H-8), 1.88 (m, 1H, H-6), 1.04 (d, 3H, $J=6.8$ Hz, H-10); MS (FAB): m/z 662 ($M^+ + 1$). Anal. calcd for $C_{32}H_{39}NO_{14}$: C, 58.09; H, 5.90; N, 2.11. Found: C, 58.30; H, 6.10; N, 2.31%; [α] −65.38 (c 0.26 in methanol).

Compound 6. Yield: 45%; mp 183 °C; IR (KBr): 1748 cm^{-1} (>Cb=O), 1678 cm^{-1} (>C=O); ^1H NMR (200 MHz $CDCl_3$): δ 7.24 (m, 1H, Ar–H) 7.16 (s, 1H, H-3) 7.08 (bs, 1H, Ar–H) 6.95 (d, 1H, $J=8$ Hz, Ar–H) 6.67 (d, 1H, $J=8$ Hz, Ar–H) 5.29–4.85 (m, 6H, H-1′–H-4′, H and H-7) 4.33 (dd, 1H, $J=12$, 4 Hz, H-6′) 4.14 (dd, 1H, $J=12$, 2 Hz, H-6′) 3.80 (s, 3H, OCH_3) 3.73 (m, 1H, H-5′) 3.11 (m, 1H, H-5) 2.35 (m, 1H, H-6) 2.29 (m, 1H, H-9) 2.25 (m, 1H, H-8), 2.15 (m, 1H, H-6), 2.10, 2.04, 2.03, 1.99, 1.89 (s, 3H each, $-COCH_3$). MS (FAB): m/z 692 ($M^+ + 1$). Anal. calcd for $C_{33}H_{41}NO_{15}$: C, 57.30; H, 5.93; N, 2.02. Found: C, 57.43; H, 6.08; N, 2.22%.

Table 2. In-utero evaluation of compounds designed for anti-adhesive activity in hamster[a]

Compound	Quantity[a] (μL)	Animal n	Pregnancy status (mean ± SE)				Comments
			Implantation		Corpora lutea		
			Treated horn (TH)	Control horn (CH)	Treated horn (TH)	Control horn (CH)	
4	10.00	2	2.9±0.7	2.1±0.5	2.04±0.6	2.1±0.5	TH and CH—Normal implants
	20.00	2	1.8±0.6	2.1±0.6	1.8±0.5	2.3±0.4	TH—Resorption; CH—Normal implants
6	10.00	2	0.00	0.00	3.2±0.8	2.9±0.7	TH and CH—No implants
	20.00	2	3.5±1.2	0.00	4.6±1.1	4.7±1.1	TH—Black implants; CH—No implants
7	10.00	2	0.00	0.00	2.9±0.8	2.4±0.9	TH and CH—No implants
	20.00	2	1.60±1.0	2.10±0.6	2.6±0.7	2.1±0.8	TH—Resorption; CH—Normal implants
8	10.00	3	1.10±0.07	1.90±0.08	2.3±0.7	2.1±0.8	TH—Resorption; CH—Normal implants
	20.00	2	1.00±0.5	0.00	2.5 1.0	2.1±0.7	TH—Resorption; CH—Normal implants
10	10.00	2	2.40±0.7	1.40±0.5	1.6±0.8	1.5±0.4	TH and CH—Normal implants
	20.00	2	2.1±0.7	1.7±0.4	2.3±0.8	1.7±0.3	TH&—Resorption; CH—Normal implants

[a]Concentration: 1 μg/μL.

Table 3. In-vivo evaluation of compounds designed for anti-adhesive activity in hamster

Compound no	Dose (mg/kg)	Animal n	Pregnancy status (mean ± SE)		Comments
			Implantation	Corpora lutea	
Vehicle	—	4	8.12±3.6	8.67±4.8	Normal implants
4	10.00	3	6.00±0	6.234±1.2	Normal implants
6	10.00	3	6.47±2.8	6.59±4.9	Normal implants
7	10.00	3	3.48±1.9	3.96±3.6	50% Resorption
8	10.00	2	1.69±0.7	1.98±0.8	Complete resorption
10	10.00	4	6.28±4.7	6.29±5.6	Normal implants

Compound 7. Yield: 56%; mp 186 °C; IR (KBr): 1758 cm^{-1} (>C=O), 1642 cm^{-1} (>C=O); ^1H NMR (200 MHz $CDCl_3$): δ 7.38 (dd, 2H, J = 6.8, 2 Hz, Ar–H), 7.16 (s, 1H, H-3), 7.06 (s, 1H, NH exchangeable), 6.85 (dd, 2H, J = 6.8, 2 Hz, Ar–H), 5.29–4.89 (m, 6H, H-1′–H-4′, H-1 and H-7), 4.32 (dd, 1H, J = 12, 4 Hz, H-6′), 4.14 (dd, 1H, J = 12, 2 Hz, H-6′), 3.79 (s, 3H, OCH_3), 3.75 (m, 1H, H-5′), 3.12 (m, 1H, H-5), 2.29 (m, 1H, H-6), 2.19 (m, 1H, H-9), 2.10, 2.04, 2.03, 2.01, 1.92 (s, 3H each, $COCH_3$), 1.99 (m, 1H, H-8), 1.95 (m, 1H, H6). 1.04, (d, 3H, J = 6.8 Hz, H-10). MS (FAB): m/z 692 ($M^+ + 1$). Anal. calcd for $C_{33}H_{41}NO_{15}$: C, 57.30; H, 5.93; N, 2.02. Found: C, 57.33; H, 5.72; N, 2.22; [α] −64.23 (c 0.26 in methanol).

Compound 8. Yield: 40%; mp 67 °C; IR (KBr): 1752 cm^{-1}, 1642 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$); δ 7.43–7.22 (m, 5H, Ar–H), 7.15 (s, 1H, H-3), 5.77 (d, 1H, J = 7.2 Hz, NH exchangeable), 5.29 (d, 1H, J = 2.4 Hz, H-1), 5.26–4.94 (m, 5H, H-1′–H-4′ and H-7), 4.50 (dd, 1H, J = 12, 4 Hz, H-6′), 4.20 (dd, 1H, J = 12, 2 Hz, H-6′), 3.92 (s, 3H, OCH_3), 3.86 (m, 2H, H-5′ and $CHCH_2$), 3.35 (dd, 1H, J = 13.8, 6 Hz, $CHCH_2$), 3.25 (dd, 1H, J = 13.8, 6 Hz, $CHCH_2$), 3.08 (m, 1H, H-5), 2.36 (m, 1H, H-6), 2.24, 2.17, 2.15, 2.13, 2.10 (s, 3H each, $COCH_3$), 2.07 (m, 1H, H-9), 2.05 (m, 1H, H-8), 1.57 (m, 1H, H-6), 1.21 (d, 3H, J = 6.8 Hz, H-10). MS (FAB): m/z 748 ($M^+ + 1$), 770 ($M^+ + Na$). Anal. calcd for $C_{36}H_{45}NO_{16}$: C, 57.83; H, 6.02; N, 1.87. Found: C, 57.77; H, 6.26; N, 1.88%; [α] −99.2 (c 0.25 in methanol).

Compound 9. Yield: 53.6%; mp 105 °C; IR (KBr): 3486 cm^{-1} (NH), 1750 cm^{-1}, 1640 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 8.29 (bs, 1H, NH exchangeable), 7.53 (d, 1H, J = 8, H-4″), 7.37 (d, 1H, J = 8 Hz, H-7″), 7–7.14 (m, 3H, H-3, H-2″ and H-6″), 6.99 (m, 1H, H-5″), 5.72 (d, 1H, J = 7.4 Hz, H-1), 5.22–4.77 (m, 5H, H-2′–H-4′ H-1 and H-7), 4.30 (dd, 1H, J = 12, 4, H-6′), 4.17 (m, 1H, $CHCOOCH_3$), 4.11 (dd, 1H, J = 12, 2 Hz, H-6′), 3.74 (s, 3H, OCH_3), 3.70 (m, 1H, H-5′), 3.34 (m, 2H, $CH_2CHCOOCH_3$), 2.80 (m, 1H, H-5), 2.08 (m, 1H, H-6), 2.08 (m, 1H, H-9), 2.03, 2.01, 2.0, 1.96, 1.89 (s, 3H each, $COCH_3$), 1.85 (m, 1H, H-8), 1.83 (m, 1H, H-6), 0.96 (d, 3H, J = 6.8 Hz, H-10). MS (FAB): m/z 787 ($M^+ + 1$). Anal. calcd for $C_{38}H_{46}N_2O_{16}$, H_2O: C, 56.71; H, 5.97; N, 3.48. Found C, 56.70; H, 6.03; N, 3.58%; [α] −80.78 (c 0.25 in methanol).

Compound 10. Yield: 62.7%; mp 185 °C; IR (KBr): 3482 cm^{-1} (OH), 1754 cm^{-1}, 1662 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.03 (s, 1H, H-3), 6.95 (d, 2H, J = 8.4 Hz, Ar–H), 6.77 (d, 2H, J = 8.4 Hz, Ar–H), 6.1 (bs, 1H, OH exchangeable), 5.66 (d, 1H, J = 7.3 Hz, NH exchangeable), 5.22–4.78 (m, 6H, H-1′, H-4′, H-1 and H-7), 4.25 (dd, 1H, J = 12, 4 Hz, H-6′), 4.16 (m, 1H, $CHCH_2$), 4.15 (dd, 1H, J = 12, 2 Hz, H-6′), 3.77 (s, 3H, OCH_3), 3.77 (m, 1H, H-5′), 3.2 (dd, 1H, J = 13, 5 Hz, $CHCH_2$), 2.9 (d, 1H, $CHCH_2$), 2.88 (m, 1H, H-5), 2.15 (m, 2H, H-6, H-9), 2.09, 2.05, 2.04, 2.03, 2.0 (s, 3H each, $COCH_3$), 2.03 (m, 2H, H-8, H-6), 0.99 (d, 3H, J = 6.8 Hz, H-10). MS (ES): m/z 764 ($M^+ + 1$). Anal. calcd for $C_{36}H_{46}NO_{17}.1/2H_2O$: C, 55.95; H, 6.08; N, 1.8. Found: C, 55.80; H, 6.2; N, 2.01%; [α] −102.74 (c 0.25 in methanol).

Compound 11. Yield: 60%; mp 75 °C; IR (KBr): 3428 cm^{-1} (NH), 1752 cm^{-1}, 1664 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.14 (s, 1H, H-3), 5.89 (m, 1H, NH exchangeable), 5.23–4.83 (m, 6H, H-1′–H-4′, H-1 and H-7), 4.25 (dd, 1H, J = 12, 4 Hz, H-6′), 4.11–4.03 (m, 3H, H-6′ and NCH_2), 3.76 (s, 3H, OCH_3), 3.76 (m, 1H, H-5′), 3.01 (m, 1H, H-5), 2.28 (m, 1H, H-6), 2.24 (m, 1H, H-9), 2.20 (m, 1H, H-8), 2.09, 2.04, 2.03, 1.99, 1.91 (s, 3H each, $COCH_3$), 1.85 (m, 1H, H-6), 1.03 (d, 3H, J = 6.8, H-10). MS (ES): m/z 624 ($M^+ + 1$). Anal. calcd for $C_{29}H_{37}NO_{14}.3/2H_2O$: C, 53.53; H, 6.15; N, 2.15. Found: C, 53.26; H, 5.93; N, 2.19%.

Compound 12. Yield: 81%; mp 80 °C; IR (KBr): 1752 cm^{-1}, 1662 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.18 (m, 10H, Ar–H), 7.0 (s, 1H, H-3), 6.19 (d, 1H, J = 7.5 Hz, NH exchangeable), 5.22–4.83 (m, 6H, H-1′-H-4′, H-1 and H-7), 4.30 (dd, 1H, J = 12, 4 Hz, H-6′), 4.16 (dd, 1H, J = 12, 2 Hz, H-6′), 3.95 (m, 1H, $CHCH_2$), 3.7 (m, 1H, H-5′), 3.29 (m, 2H, NCH_2), 2.8 (m, 1H, H-5), 2.30 (m, 2H, CH_2CH), 2.24 (m, 1H, H-6), 2.09, 2.04, 2.03, 2.0, 1.88 (s, 3H each, $COCH_3$), 1.94 (m, 1H, H-9), 1.92 (m, 1H, H-8), 1.81 (m, 1H, H-6), 1.01 (d, 3H, J = 6.8 Hz, H-10). MS (ES): m/z 780 ($M^+ + 1$), 802 ($M^+ + Na$). Anal. calcd for $C_{41}H_{49}NO_{14}$: C, 63.15; H, 6.29; N, 1.79. Found: C, 63.29; H, 6.37; N, 2.08%; [α] −73.96 (c 0.26 in methanol).

Compound 13. Yield: 88%; mp 69 °C; IR (KBr): 1754 cm^{-1}, 1654 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.09 (s, 1H, H-3), 5.24 (m, 1H, NH exchangeable), 5.18–4.82 (m, 6H, H-1′-H-4′, H-1 and H-7), 4.31 (dd, 1H, J = 12, 4 Hz, H-6′), 4.13 (dd, 1H, J = 12, 2 Hz, H-6′), 3.75 (m, 1H, H-5′), 3.31 (m, 2H, NCH_2), 2.96 (m, 1H, H-5), 2.2 (m, 1H, H-6), 2.09, 2.04, 2.03, 2.0, 1.92 (s, 3H each, $COCH_3$), 1.81 (m, 1H, H-9), 1.75 (m, 1H, H-8), 1.67 (m, 1H, H-6), 1.39 (m, 4H, $(CH_2)_2$), 1.02 (d, 3H, J = 6.8, H-10), 0.96 (t, 3H, J = 7, CH_2CH_3); MS (ES): m/z 642 ($M^+ + 1$); Analysis calcd for $C_{30}H_{43}NO_{14}$: C, 56.10; H, 6.70; N, 2.18. Found: C, 55.87; H, 6.71; N, 2.38%; [α] −58.07 (c 0.26 in methanol).

Compound 14. Yield: 83%; mp: 105 °C; IR (KBr): 1752 cm^{-1} (>C=O), 1674 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.31 (m, 5H, Ar–H), 7.13 (s, 1H, H-3), 5.65 (m, 1H, NH exchangeable), 5.23–4.82 (m, 6H, H-1′-H-4′, H-1 and H-7), 4.48 (m, 2H, NCH_2), 4.28 (dd, 1H, J = 12, 4 Hz, H-6′), 4.10 (dd, 1H, J = 12 and 2 Hz, H-6′), 3.70 (m, 1H, H-5′), 2.9 (m, 1H, H-5), 2.17 (m, 1H, H-9), 2.09 (m, 1H, H-8), 2.17, 2.09, 2.02, 2.01, 1.99 (s, 3H each, $COCH_3$), 1.02 (d, 3H, J = 6.8 Hz, H-10). MS (ES): m/z 676 ($M^+ + 1$), 698 ($M^+ + Na$). Anal. calcd for $C_{33}H_{41}NO_{14}$; C, 58.66; H, 6.07; N, 2.07. Found: C, 58.66; H, 5.93; N, 2.10%; [α] −60.76 (c 0.26 in methanol).

Compound 15. Yield: 61%; mp: 101 °C; IR (KBr): 1742 cm^{-1} (>C=O), 1642 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.13 (s, 1H, H-3), 5.88 (d, 1H, J = 8.5 Hz, NH exchangeable), 5.23–4.83 (m, 6H, H-1′-

H-4′, H-1 and H-7), 4.6 (m, 1H, CHCOOCH$_3$), 4.33 (dd, 1H, $J=12$, 4 Hz, H-6′), 4.14 (dd, 1H, $J=12$, 2 Hz, H-6), 3.74 (s, 3H, OCH$_3$), 3.74 (m, 1H, H-5), 3.05 (m, 1H, H-5), 2.29–2.15 (m, 3H, H-6, H-9 and CH(CH3)2), 2.13, 2.09, 2.04, 2.03, 1.94 (s, 3H each, COCH$_3$), 1.83 (m, 1H, H-8), 1.77 (m, 1H, H-6), 1.03 (d, 3H,$J=6.6$, H-10), 0.92 (m, 6H, CH(CH$_3$)$_2$). MS (FAB): m/z 700 ($M^+ + 1$). Anal. calcd for $C_{32}H_{45}NO_{16}$: C, 54.93; H, 6.43; N, 2.00. Found: C, 55.05; H, 6.28; N, 2.14%; [α] −87.84 (c 0.25 in methanol).

Compound 16. Yield: 80%; Viscous; IR (KBr): 1748 cm^{-1} (>C=O); ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.18–6.77 (m, 5H, H-3, Ar), 5.23–4,85 (m, 6H, H^{-1}, H-7 and H-1′-H-4′), 4.27 (dd, 1H, $J=12$, 4 Hz, H-6′), 4.18 (dd, 1H, 1H, $J=12$, 2 Hz, H-6′), 3,70 (m, 4H, 2×NCH$_2$), 3.22 (m, 5H, 2×NCH$_2$ and H-5′), 2.67 (m, 1H, H-5), 2.35 (m, 1H, H-6), 2.20 (m, 1H, H-9), 2.14, 2.11, 2.09, 2.03, 2.01 (s, 3H each, COCH$_3$), 1.93 (m, 1H, H-8), 1.86 (m, 1H, H-6), 1.03 (d, 3H, $J=6.8$ Hz, H-10); MS (ES): m/z 765 ($M^+ + 1$). Anal. calcd for $C_{36}H_{45}N_2O_{14}$.Cl: C, 56.54; H, 5.89; N, 3.66. Found: C, 56.52; H, 5.70; N, 3.60%.

Compound 17. Yield: 86.7%; mp 105 °C; IR (KBr): 1750 cm^{-1} 1624 (>C=O); ^{1}H NMR (200 MHz, CDCl$_3$): δ 6.91 (m, 5H, Ar-H), 6.45 (s, 1H, H-3), 5.23–4.85 (m, 6H, H-1′–H-4′, H-1 and H-7), 4.30 (dd, 1H, $J=12$, 4 Hz, H-6′), 3.74–3.69 (m, 4H, H-5′, H-5 and NCH$_2$), 3.15 (m, 4H, N(CH$_2$)$_2$), 2.20 (m, 1H, H-6), 2.14, 2.10, 2.10, 2.03, 2.01 (s, 3H each, COCH$_3$), 1.95 (m, 1H, H-9), 1.93 (m, 1H, H-8), 1.87 (m, 1H, H-6), 1.02 (d, 3H, $J=6.8$ Hz, H-10); MS (ES): m/z 731 ($M^+ + 1$). Anal. calcd for $C_{36}H_{46}N_2O_{14}$: C, 59.17; H, 6.30; N, 3.8. Found: C, 59.13; H, 6.44; N, 3.5%.

Amidation of loganic acid

Method A. Representative procedure for the synthesis of compounds **18**–**20**. To a stirred solution of **10** (500 mg, 0.65 mmol) in dry methanol (25 mL) was added sodium metal (15 mg) and stirring continued for 4–6 h at room temperature. After complete deacetylation, the reaction mixture was passed through amberlite IR-120 (H$^+$) (500 mg) column and eluted with methanol (35 mL). Concentration of the eluent in vacuo gave crude product which was purified by column chromatography, using methanol/chloroform (10:90, v/v), to afford a white amorphous solid (300 mg).

Method B

This procedure was employed for the synthesis of compound **19**. To a solution of loganic acid **2** (200 mg, 0.53 m mol), *p*-anisidine (65 mg, 0.53 m mol) and triethylamine (0.2 mL) in water (1.5 mL) was added, 1-ethyl-3-(3′-dimethylaminopropyl carbondiimide)hydrochloride, (EDCI) (121 mg, 0.63 mmol) dissolved in water (1.5 mL) at room temperature. After completion of the reaction, solvent was evaporated in vacuo. Reaction mixture was dissolved in methanol and purified by column chromatography, using methanol: chloroform, (12:88, v/v), to give white amorphous solid (160 mg).

Method C. Following this procedure, compounds **18**–**20** were synthesised. To a solution of loganic acid **2** (200 mg, 0.52 m mol), (benzotriazol-1-yloxy) tris-(dimethylamino) phosphonium hexafluorophosphate (BOP) (282 mg, 0.62 mmol) and *N*,-*N*-diisopropyl ethylamine (1 mL, 0.76 mmol) in dry DMF (4 mL) was added phenyl alanine (257 mg, 1.19 mmol) and the mixture was stirred for 3–4 h at room temperature. After completion of the reaction, solvent was evaporated in vacuo and the obtained thick mass was dissolved in butanol. The solution was washed successively with 3 N HCl (3×1.5 mL), saturated solution of sodium bicarbonate (3×1.5 mL) and brine. It was dried over anhydrous sodium sulphate, filtered and the filterate concentrated. The crude product was purified by column chromatography using methanol/chloroform (12:88, v/v) to give 140 mg of the desired product.

Compound 18. Yield: 86%; mp 80 °C; IR (neat): 3738 cm^{-1} (OH), 1672 cm^{-1} (>C=O); ^{1}H NMR (200 MHz, D$_2$O): δ 7.30 (m, 5H, Ar-H), 7.0 (s, 1H, H-3), 5.25 (m, 1H, H^{-1}), 4.65 (m, 1H, H-1′), 4.05 (m, 1H, H-7), 3.75 (m, 1H, H-6′), 3.55 (m, 1H, H-6′), 3.3–3.12 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.10 (m, 1H, H-5), 2.12 (m, 1H, H-6), 2.10 (m, 1H, H-9), 1.85 (m, 1H, H-8), 1.75 (m, 1H, H-6), 0.99 (m, 3H, H-10). MS (ES): m/z 474 ($M^+ + Na$). Anal. calcd for $C_{22}H_{29}NO_9$, 31/2H$_2$O: C, 51.36; H, 7.00; N, 2.72. Found: C, 51.55; H, 6.94; N, 3.0%.

Compound 19. Yield: 63%; mp 71 °C; IR (KBr): 3728 cm^{-1} (OH), 1636 cm^{-1} (>C=O); ^{1}H NMR (200 MHz, D$_2$O): δ 7.12 (d, 2H, $J=9$ Hz, Ar-H), 6.97 (s, 1H, H-3), 6.81 (d, 2H, $J=9$ Hz, Ar–H), 5.26 (d, 1H, $J=2.7$ Hz, H-1), 4.67 (m, 1H, H-1′), 4.14 (bs, 1H, H-7), 3.74 (m, 1H, H-6′), 3.64 (s, 3H, OCH$_3$), 3.53 (dd, 1H, $J=12$, 4.5 Hz, H-6′), 3.34–3.04 (m, 4H, H-2′, H-3′, H-4′ and H-5′), 3.19 (m, 1H, H-5), 2.16 (m, 1H, H-6), 2.10 (m, 1H, H-9), 1.95 (m, 1H, H-8), 1.80 (m, 1H, H-6), 0.89 (d, 3H, $J=6.6$ Hz, H-10). MS (ES): m/z 504 ($M^+ + Na$). Anal. calcd for $C_{23}H_{31}NO_{10}$.11/2H$_2$O: C, 54.33; H, 6.69, N, 2.75. Found: C, 54.57; H, 6.72; N, 2.99%; [α] −85.28 (c 0.26 in methanol).

Compound 20. Yield: 83%; mp 120 °C (dec.); IR (neat): 3758 cm^{-1} (OH); ^{1}H NMR (200 MHz, D$_2$O): δ 7.11 (m, 2H, Ar–H), 6.98 (s, 1H, H-3), 6.86 (m, 2H, Ar–H), 5.35 (bs, 1H, H-1), 4.74 (m, 2H, H-1′, CHCOOCH$_3$), 4.00 (m, 2H, H-6′), 3.86 (m, 1H., H-7), 3.73 (s, 3H, OCH$_3$), 3.73 (m, 1H, H-5′), 3.28 (m, 4H, H-2′, H-3′, H-4′, Ar–CH$_2$), 2.98 (m, 1H, Ar–CH$_2$), 2.97 (m, 1H, H-5), 2.07 (m, 1H, H-6), 1.82 (m, 1H, H-9), 1.77 (m, 1H, H-8), 1.40 (m, 1H, H-6), 0.99 (bs, 3H, H-10). MS (ES): m/z 576 ($M^+ + Na$). Anal. calcd for $C_{26}H_{35}NO_{12}2H_2O$: C, 52.97; H, 61.28; N, 2.37. Found C, 52.93; H, 6.45; N, 2.37%.

Enzymatic hydrolysis of loganin with β-glucocidase (21)

To a solution of loganin (1.0 gm) in mixture of citrate buffer (20 mL, pH-5) and water (20 mL) was added β-gluosidase (50 mg). Reaction mixture was kept at 30 °C for 24 h. After completion of the reaction, the mixture was extracted with ether, dried over anhydrous

sodium sulphate, concentrated to a thick mass and purified by column chromatography using methanol/chloroform (1:99, v/v) to give the desired product **21** (550 mg).

Yield: 92%; oil; IR (neat): 3686 cm^{-1} (OH), 1702 cm^{-1} (>C=O); ^1H NMR (200 MHz $CDCl_3$): δ 7.41 (s, 1H, H-3), 5.39 (d, 1H, J = 2.08 Hz, H-1α, 8%), 4.98 (d, 1H, J = 5 Hz, H-1β, 92%), 4.14 (m, 1H, H-7), 3.71 (s, 3H, OCH_3), 3.19 (m, 1H, H-5), 2.3 (m, 1H, H-6), 2.09 (m, 1H, H-9), 1.90 (m, 1H, H-8), 1.65 (m, 1H, H-6), 1.14 (d, 3H, J = 6.6, H-10). MS (EI): m/z 228 (molecular ion); Analysis calcd for $C_{11}H_{16}O_5.1/2H_2O$: C, 55.69; H, 7.17. Found: C, 55.59; H, 7.15%.

Generation of ether linkage (22–26). To a stirred solution of Loganin aglucone (100 mg) in respective alcohols (5 mL) was added BF_3OEt_2 [0.25 mL] at 0 °C. Stirring was continued for 2–4 h. Solvent was evaporated, the reaction mixture was dissolved in ether and the solution was successively washed with water to remove BF_3–OEt_2. The crude product was purified by column chromatography, eluting with ethylacetate/hexane (25:75, v/v), to yield the desired compound (92 mg).

Compound 22. Yield 86.7%; oil; IR (neat): 1702 cm^{-1} (>C=O); ^1H NMR (200 MHz, $CDCl_3$); δ 7.38 (s, 1H, H-3), 4.91 (d, 1H, J = 2.8 Hz, H-1α, 15.7%), 4.62 (d, 1H, J = 4 Hz, H-1β, 84.3%), 4.10 (m, 1H, H-7), 3.71 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.12 (m, 1H, H-5), 2.28 (m, 1H, H-6α), 2.02 (m, 1H, H-9), 1.82 (m, 1H, H-8), 1.68 (m, 1H, H-6β), 1.16 (d, 3H, J = 6.8 Hz, H-10). MS (EI): 242 (molecular ion). Anal. calcd for $C_{12}H_{18}O_5$: C, 59.50; H, 7.04. Found: C, 59.52; H, 7.43%.

Compound 23. Yield: 87%; oil; IR (neat): 1700 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.40 (s, 1H, H-3), 5.0 (d, 1H, J = 3 Hz, H = 1α, 16%), 4.67 (d, 1H, J = 4.8 Hz, H-1β, 84%), 4.12 (t, 1H, J = 4.4 Hz, H-7), 3.88 (m, 1H, OCH_2), 3.71 (s, 3H, OCH_3), 3.58 (m, 1H, OCH_2), 3.14 (m, 1H, H-5), 2.24 (m, 1H, H-6), 2.00 (m, 1H, H-8), 1.82 (m, 1H, H-9), 1.59 (m, 1H, H-6), 1.25 (m, 3H, CH_3), 1.12 (d, 3H, J = 6.8 Hz, H-10). MS (EI): m/z 256 (molecular ion). Anal. calcd for $C_{13}H_{20}O_5$: C, 60.93; H, 7.81. Found: C, 60.92; H, 7.94%.

Compound 24. Yield: 93.6%; oil; IR (neat): 1700 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.49 (s, 1H, H-3), 4.99 (d, 1H, J = 2.95, H-1α, 16.4%), 4.64 (d, 1H, J = 4.9 Hz, H-1β, 83.6%), 4.11 (t, 1H, J = 4.4 Hz, H-7), 3.78 (m, 1H, OCH_2), 3.71 (s, 3H, OCH_3), 3.45 (m, 1H, OCH_2), 3.14 (m, 1H, H-5), 2.26 (m, 1H, H-6), 2.01 (m, 1H, H-9), 1.83 (m, 1H, H-8), 1.58 (m, 2H, –$OCH_2C\underline{H}_2$), 1.58 (m, 1H, H-6), 1.12 (d, 3H, J = 6.8 Hz, H-10). MS (EI): m/z 270 (molecular ion). Anal. calcd for $C_{14}H_{22}O5$: C, 62.22; H, 8.1. Found: C, 62.14; H, 8.3%.

Compound 25. Yield: 63.7%; oil; IR (neat): 1702 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.37 (s, 1H, H-3), 5.05 (d, 1H, J = 2.9 Hz, H-1α, 7.4%), 4.75 (d, 1H, J = 4.5 Hz, H-1β, 92.6%), 4.10 (t, 1H, J = 4.3 Hz, H-7), 3.89 (m, 1H, OCH_2), 3.75 (m, 1H, OCH_2), 3.70 (s, 3H, OCH_3), 3.56 (t, 2H, J = 4.6, $OCH_2C\underline{H}_2$), 3.38 (s, 3H, OCH_3), 3.16 (m, 1H, H-5), 2.26 (m, 1H, H-6), 2.07 (m, 1H, H-9), 1.85 (m, 1H, H-8), 1.60 (m, 1H, H-6), 1.12 (d, 3H, J = 6.8 Hz, H-10). MS (EI): m/z 286 (molecular ion). Anal. calcd for $C_{14}H_{22}O_6$: C, 58.74; H, 7.6. Found: C, 58.48; H, 7.6%.

Compound 26. Yield: 65%; oil; IR (neat): 1700 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.37 (s, 1H, H-3), 5.07 (d, 1H, J = 3.1 Hz, H-1α, 19%), 4.73 (d, 1H, J = 4.7 Hz, H-1β, 81%), 4.12–4.02 (m, 2H, H-7, OCH_2), 3.77 (m, 1H, OCH_2), 3.71 (s, 3H, OCH_3), 3.65 (m, 2H, CH_2Cl), 3.13 (m, 1H, H-5), 2.27 (m, 1H, H-6), 2.05 (m, 2H, H-9 and H-8), 1.74 (m, 1H, H-6). MS (EI): m/z 290 (molecular ion). Anal. calcd for $C_{13}H_{19}O_{15}Cl.1/2H_2O$: C, 52.17; H, 6.6. Found: C, 52.16; H, 6.72%.

Basic hydrolysis of aglucone methyl ether

Compound 27. To a solution of aglucone methyl ether **22** (1.0 g, 4.38 m mol) in methanol (15 mL) was added NaOH (2 N, 1 mL) and stirred for 4 h at room temperature. After completion of the reaction, the mixture was neutralised with dil hydrochloric acid solution (15 mL), and extracted with ethyl acetate. The solution was dried over anhydrous sodium sulphate and concentrated to give the expected product (720 mg).

Yield: 76.5%; oil; IR (neat): 1688 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$) δ 7.49 (s, 1H, H-3), 4.93 (d, 1H, J = 2.92 Hz, H-1α, 10%), 4.64 (d, 1H, J = 4.2 Hz, H-1β, 90%), 4.12 (t, 1H, J = 4.6 Hz, H-7), 3.50 (s, 3H,–OCH_3), 3.09 (m, 1H, H-5), 2.29 (m, 1H, H-6), 2.07 (m, 1H, H-9), 1.82 (m, 1H, H-8), 1.72 (m, 1H, H-6), 1.12 (d, 3H, J = 6.8 Hz, H-10). MS (EI): m/z 228 (molecular ion). Anal. calcd for $C_{11}H_{16}O_5$: C, 57.89; H, 7.01. Found: C, 57.70; H, 7.2%.

HOBT ester of aglucone carboxylic acid (28)

The same procedure was employed as in the case of compound **4**.

Yield: 56%; oil; IR (neat): 1778 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$); δ 8.05 (m, 1H, Ar–H), 7.86 (s, 1H, H-3), 7.57–7.37 (m, 3H, Ar–H), 5.10 (d, 1H, J = 2.93 Hz, H-1α, 23.4%), 4.81 (d, 1H, J = 4 Hz, H-1β, 76.6%), 4.19 (m, 1H, H-7), 3.57 (s, 3H, OCH_3), 3.26 (m, 1H, H-5), 2.31 (m, 1H, H-6), 1.86 (m, 2H, H-9 and H-8), 1.63 (m, 1H, H-6), 1.16 (d, 3H, J = 6.8 Hz, H-10). MS (EI): m/z 345 (molecular ion); Anal. calcd for $C_{17}H_{19}N_3O_5$: C, 59.13; H, 58.83; N, 12.17. Found: C, 58.83; H, 5.75; N, 11.88%.

Amide of aglucone. The same procedure as given for compounds **5**–**16** was followed.

Compound 29. Yield: 58%; oil; IR (neat): 1632 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.40 (d, 2H, J = 8.5, Ar–H), 7.30 (s, 1H, H-3), 7.05 (s, 1H NH exchangeable), 4.64 (d, 1H, J = 3.4 Hz, H-1α, 13%), 4.61 (d, 1H, J = 4.0 Hz, H-1β, 87%), 4.16 (t, 1H, J = 4 Hz, H-7), 3.78 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.20 (m, 1H, H-5), 2.26 (m, 1H, H-6), 2.12 (m, 1H, H-9), 1.89 (m, 1H, H-8), 1.75 (m, 1H, H-6), 1.12 (d, 3H, J = 6.8 Hz, H-10).

MS (EI): *m/z* 333 (molecular ion). Anal. calcd for $C_{18}H_{23}NO_5$: C, 64.86; H, 6.90; N, 4.20. Found: C, 64.57; H, 7.10; N, 4.38%.

Compound 30. Yield: 63%; oil; IR (neat): 1654 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.53–7.06 (m, 5H, Ar–H), 7.21 (s, 1H, H-3), 4.92 (d, 1H, $J=3.9$ Hz, H-1α, 13.5%), 4.64 (d, 1H, $J=4.4$ Hz, H-1β, 86.5%), 4.11 (bs, 1H, H-7), 3.50 (s, 3H, OCH_3), 3.23 (m, 1H, H-5), 2.25 (m, 1H, H-6), 2.14 (m, 1H, H-9), 1.83 (m, 1H, H-8), 1.74 (m, 1H, H-6), 1.13 (d, 3H, $J=6.8$ Hz, H-10). MS (EI): *m/z* 303 (molecular ion). Anal. calcd for $C_{17}H_{18}NO_{14}$: C, 67.32; H, 5.94; N, 4.6. Found: C, 67.46; H, 6.01; N, 4.83%.

Synthesis of methyl-3,6-anydro-α-D-glucopyranoside (32)

To a stirred solution of 6-tosyl α-D-glucopyranoside **31** (1 g, 2.87 m mol) in ethanol (10 mL) was added 1N NaOH (2 mL) and the mixture heated at 60 °C for 2 h. After completion of the reaction, the mixture was neutralised with drops of acetic acid and evaporated to dryness in vacuo to give a white crude product which was dissolved in hot acetone. Insoluble white solid was filtered off. The filtrate was concentrated to give a gummy substance which was purified by column chromatography, using methanol/chloroform (1:99, v/v) as eluent. The pure product (420 mg) was viscous in nature.

Yield: 83%; viscous: IR (neat): 3436 cm^{-1} (OH); ^1H NMR (300 MHz, $CDCl_3$): δ 5.07 (d, 1H, $J=4.5$ Hz, H-1), 4.63 (m, 2H, H-3 and H-5), 4.45 (m, 1H, H-4), 4.17 (m, 1H, H-2), 4.15 (m, 1H, H-6), 3.9 (m, 1H, H-6), 3.5 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 97.28 (C-1), 75.52 (C-3), 72.22 (C-5) 70.80 (C-4). 70.39 (C-2), 68.88 (C-6), 56.96 (OCH_3). MS (EI): *m/z* 176 (molecular ion). Anal. calcd for $C_7H_{12}O_5$: C, 47.72; H, 6.81. Found: C, 47.60; H, 6.80%.

Methyl-2,4-di-0-cinnamoyl-3,6-anhydro-α,D-glucopyranoside (33). To a stirred solution of 3,6-anhydro methyl α-D-glucopyranoside **32** (200 mg, 1.12 mmol), pyridine (0.2 mL, 2.49 mmol) and DMAP (10 mg) in dry ethylacetate (8 mL) was added cinnamic anhydride (1.38 g, 4.38 mmol) and the mixture was stirred at room temperature for 12 h. Subsequently, it was diluted with the same solvent and washed with saturated solution of aqueous sodium bicarbonate, followed by water. The organic layer was separated and its usual workup yielded crude product which was purified over silica gel column using methanol/chloroform (0.5; 99.5, v/v) as eluent. The product was recrystallised from ethyl acetate/hexane.

Yield: 84%; mp 179 °C; IR (KBr): 3480 cm^{-1} (OH), 1724 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$): δ 7.79 (d, 1H, $J=16$ Hz, H-α′), 7.64 (d, 1H, $J=16$ Hz, H-α″), 7.47 (m, 2H, H-1′ and H-5′), 7.39 (m, 2H, H-1″ and H-5″), 7.16 (m, 3H, H-2′, H-3′ and H-4′), 7.0 (m, 3H, H-2″, H-3″ and H-4″), 6.72 (d, 1H, $J=16$ Hz, H-β′), 6.32 (d, 1H, $J=16$ Hz, H-β″), 5.26 (m, 1H, H-1), 5.12 (m, 1H, H-3), 4.87 (m, 1H, H-2), 4.82 (m, 1H, H-4), 4.67 (bs, 1H, H-5), 4.28 (d, 1H, $J=10$ Hz, H-6α), 4.10 (dde 1H, H-6β), 3.60 (s, 3H, OCH_3). MS (EI): *m/z* 436 (molecular ion). Anal. calcd for $C_{25}H_{24}O_7.H_2O$: C, 66.07; H, 5.6. Found: C, 65.9; H, 5.3%.

References and Notes

1. Oshima, Y.; Takata, S.; Hiroshi, H.; Deyama, T.; Kinoshita, G. *J. Ethanopharmacol.* **1988**, *23*, 159.
2. Syrov, V. N.; Nobeiv, A. N.; Kushbaktov, Z. A.; Umarova, R. V. *Khim pharma. Zh.* **1990**, *24*, 40.
3. Houghton, P. J.; Hikino, H. *Planta Med.* **1989**, *55*, 123.
4. Ansari, R. A.; Aswal, B. S.; Chander, R.; Dhawan, B. N.; Garg, N. K.; Kapoor, N. K.; Kulshreshta, D. K.; Medhi, H.; Mehrotra, B. N. *Ind. J. Med. Res.* **1988**, *87*, 401.
5. Atal, C. K.; Sharma, M. L.; Kaul, A.; Khajuria, A. *J. Ethanopharmacol* **1986**, *18*, 133.
6. Simson, J. M.; t'Hart, L. A.; Van Dijk, K.; Fischer, F. C.; Desilva, K. T. D.; Labadie, R. P. *J. Ethanopharmacol.* **1989**, *26*, 169.
7. Labadie, R. P.; Vander, Nat, J. M.; Simson, J. M.; Kroes, B. H.; Kosasi, S.; Vandon, Berg, A. J. J.; t'Hart, L. A.; Vander Sluis, W. G.; Abeysekera, A.; Ramunurachchi, A.; Desilva, K. D. T. *Planta Med.* **1989**, *55*, 339.
8. Chander, R.; Dwivedi, Y.; Rastogi, R.; Sharma, S. K.; Garg, N. K.; Kapoor, N. K.; Dhawan, B. N. *Ind. J. M. Res.* **1990**, *92*, 34.
9. Garg, H. S.; Bhandari, S. P. S.; Tripathi, S. C.; Patnaik, G. K.; Puri, A.; Savena, R.; Saxena, R. P. *Phytotherapy Res.* **1994**, *8*, 224.
10. Khar, Z. K.; Mangalani, A.; Shukla, P. K.; Puri, A.; Saxena, R. P.; Tandon, J. S. *Int. J. Pharmacogn.* **1995**, *33*, 297.
11. Mathad, V. T.; Raj, K.; Bhaduri, A. P.; Sahai, R.; Puri, A.; Tripathi, L. M.; Srivastava, V. M. L. *Bioorg. Med. Chem.* **1998**, *6*, 605.
12. (a) Aplin, J. D. *J. Reprod. Fertil.* **1997**, *2*, 84. (b) Aplin, J. D.; Hey, N. A. *Biochem. Soc. Trans.* **1995**, *23*, 826. (c) CompBell, S.; Swan, H. R.; Seif, M. V.; Kimber, S. J.; Aplin, J. D. *Mol. Hum. Reprod* **1995**, *1*, 1571. (d) Hey, N. A.; Li, T.c.; Devine, P. L.; Graham, R. A.; Aplin, J. D. *Hum. Reprod.* **1995**, *10*, 2655.
13. Surveyer, G. A.; Gendler, S. J.; Pemberton, L.; Das, S. K.; Chakraborty, I.; Julian, J.; Pimental, R. A.; Wegner, C. C.; Dey, S. K.; Carson, D. D. *Endocrinology* **1995**, *136*, 3639.
14. DeSouza, M. M.; Mam, S. K.; Jalian, J.; Carson, D. D. *Biol. Reprod.* **1998**, *58*, 1503.
15. Hoffman, L. H.; Olson, G. E.; Carson, D. D.; Chilton, B. S. *Endocrinology* **1998**, *139*, 266.
16. DeLoia, J. A.; Krasnow, J. S. J.; Babaknia, A.; Julian, J.; Carson, D. D. *Hum. Reprod.* **1998**, *13*, 2902.
17. Battersby, A. R.; Kapil, R. S.; Southgate, R. *Chem. Commun.* **1968**, 131.
18. Inouye, H.; Yishida, T.; Tobila, S. *Tetrahadron Lett.* **1968**, 2945.
19. Battersby, A. R.; Hall, E. S.; Southgate, R. *J. Chem. Soc. (C)* **1969**, 721.