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Semi-synthetic analogs of pinitol as potential inhibitors of TNF-α cytokine expression in human neutrophils

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ABSTRACT

Semi-synthetic analogs of pinitol were subjected to screening by determining TNF-α expression in human neutrophils using flowcytometry. Among the tested compounds, three derivatives displayed more than 50% inhibition of TNF-α cytokine secretion in LPS induced stimulated neutrophils and can be considered as potent anti-inflammatory moieties.

Inositols and their derivatives have attracted considerable attention due to their innumerable roles in living organisms,[1] and diverse biological activities.[2] They also serve as starting material in the synthesis of natural products.[3] The major naturally occurring inositol is *myo*-inositol. Other naturally occurring isomers (occur in low concentrations) are *scyllo*-, *chiro*-, *muco*-, and *neo*-inositol. Pinitol is 3-*O*-methyl ether of D-*chiro*-inositol also known as Mateziol or Sennitol or Cathrtomannitol (Fig. 1). The configuration of D-*chiro*-inositol has been determined by Posternak,[4] although the position of the methyl group in pinitol was established by Arthur, et al.[5] in 1952, assigning its structure as 3-*O*-methyl-*chiro*-inositol.

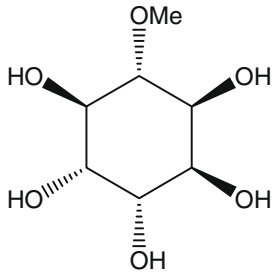

Figure 1.

Pinitol is one of the major constituents and mainly being isolated from *Bougainvillea spectabilis*,[6] *Pinus lambertiana*[7] and soyabean (*Glycine max*).[8] There is also a widespread presence of D-pinitol in Alfalfa and legumes.[9] Pinitol appears to mimic the effects of insulin by acting downstream in the insulin signaling pathway.[10] *Bougainvillea spectabilis* is used as a traditional medicine for the treatment of diabetes in Asia and West Indies.[6] Pinitol has been shown to lower the blood glucose concentration in diabetic rats and in normal rats given glucose.[11] Its hypoglycemic and anti-diabetic activities are attributed to insulin like effects in normal and alloxan induced diabetic albino mice.[12] The insulin is able to up-regulate the expression of the TNF-α gene in macrophages derived from the THP-1 cell,[13] and TNF-α is involved in insulin resistance through the inhibitory effect on insulin sensitivity.[14]

Pinitol derivatives (e.g., aminocyclitols) are reported to exhibit glycosidase inhibitory activities,[15] thus adding to their biological importance, since glycosidase inhibitors are the potential therapeutic agents.[16] Azole nucleoside analogs of D-pinitol have recently been reported as potent antitumor agents.[17] Pilot studies have indicated that co-administration of creatine (a natural nutrient found in animal foods, claimed to be an effective nutritional ergogenic aid to enhance sports related activities and physical performance[18]) with low doses of D-pinitol help in augmenting whole body creatine stores by non-caloric means.[19] The pinitol has shown its potential as anti-inflammatory agent,[20,21] and a remedy for fever and respiratory disorders.[22] Pinitol has been demonstrated to decrease the lipid laden foam cell formation in THP-1 derived human macrophages[23] and inhibit TNF-α production.[24] From the

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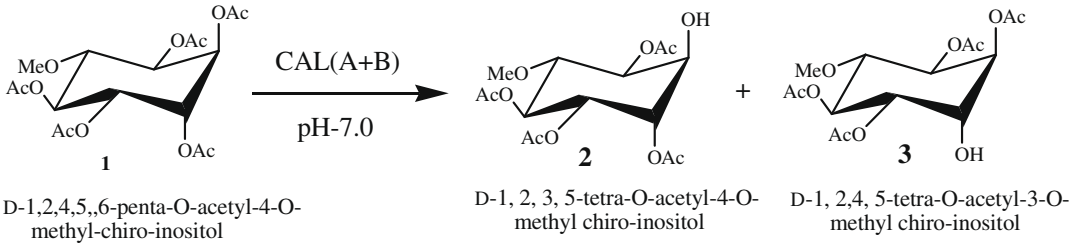
literature survey it is apparent that pinitol and its derivatives are associated with a wide range of bioactivities, for example, anti-diabetic, anti-inflammatory, anticancer, stamina enhancing. Therefore, D-pinitol analogs were subjected to study their role in inhibiting TNF-α cytokine expression in LPS activated human neutrophils, as they play essential roles in host defence through their ability to clear bacterial infections. The literature scan revealed that there are no reports of the preparation of selectively acylated and randomly/multi-acylated derivatives of pinitol. We therefore envisaged synthesizing various acylated derivatives of pinitol. In this Letter, we report the preparation of selectively acylated pinitol derivatives through (a) biotransformation, and (b) chemical approaches and their TNF-α inhibitory studies.

Enzymes are known to be the versatile catalysts for the kinetic resolution and desymmetrization of prostereogenic substrates. A variety of lipases of different origins are known to be useful in regioselective transesterification as well as hydrolysis reactions. Since it was almost impossible to get selectively acylated derivatives of pinitol through chemical transformation in one step reaction, it was envisaged that transesterification of pinitol would be an alternative process. Our attempts for the preparation of regioselectively acylated derivatives through transesterification of pinitol turned futile, though different solvent combinations, and temperature conditions were tried. Therefore, we proceeded through selective hydrolysis route for the preparation of desired compounds. In this approach, regioselective lipase catalyzed hydrolysis of penta-acylates of pinitol was studied. A number of lipases from institutional microbial repository and commercial sources were screened for the selective hydrolysis of penta-acetates, penta-butyrates and penta-benzoates of pinitol. Using the commercial lipase CAL-A+B, monodeacylated products **2** and **3** in the ratio of 48:52 were obtained by regioselective hydrolysis of D-1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-*chiro*-inositol **1** with an overall yield of 72%.[25] The reaction mixture comprising the products was separated by column chromatography and characterized as D-1,2,3,5-tetra-*O*-acetyl-4-*O*-methyl-*chiro*-inositol (**2**) and D-1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-*chiro*-inositol (**3**) on the basis of ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectral analysis (Scheme 1).

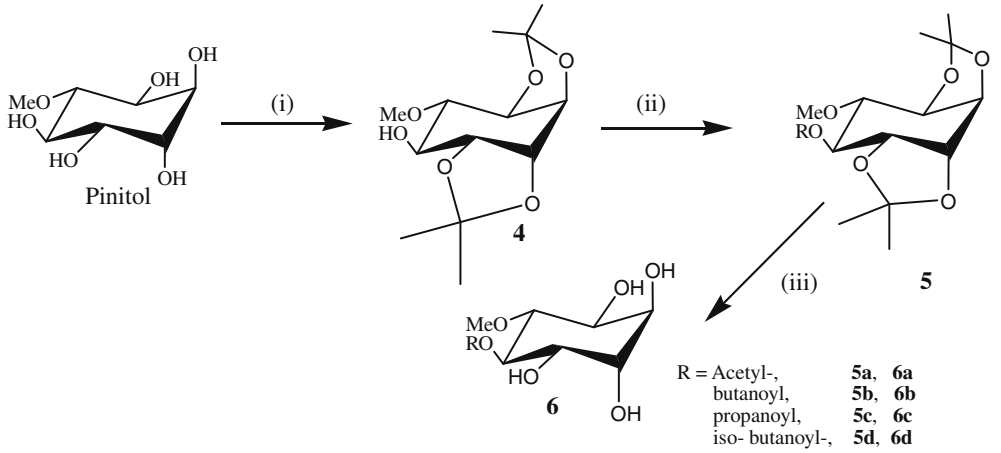
Various acyl analogs of pinitol (new as well as reported ones) were also synthesized for bioactivity screening (Schemes 2–4).[26] Preparation of 1D-3-*O*-acetyl-4-*O*-methyl-*chiro*-inositol (**6a**), 1D-3-*O*-butanoyl-4-*O*-methyl-*chiro*-inositol (**6b**), 1D-3-*O*-methyl-4-*O*-propanoyl-*chiro*-inositol (**6c**) and 1D-3-*O*-*iso*-butanoyl-4-*O*-methyl-*chiro*-inositol (**6d**) was achieved in a three step reaction process (Scheme 2). The vicinal *syn* hydroxyl functions were first protected through diacetonide formation followed by esterification of the remaining hydroxyl group by reacting it with an acid anhydride in presence of pyridine as a base to produce **5** in almost quantitative yield. Deprotection of isopropylidene acetals was achieved by a method using $HClO_4$–SiO_2.[27] Therefore, $HClO_4$–SiO_2 was prepared according to the standard procedure and used for the deprotection of isopropylidene acetals in acetonitrile. Catalytic amount of the reagent (0.035 mmol/mmol of diacetonide) was sufficient to cleave the diacetonide in half an hour to furnish the products **6 (a–d)** in 90–92% yield.

Alkoxy derivatives **7a–c** were synthesized from a common diacetonide intermediate **4**, by performing standard alkylation reactions, respectively, in presence of a base. Removal of the acetonide groups under acidic conditions described as above yielded the desired derivatives **8a–c**, respectively (Scheme 3; Joanne B. H. 2004).[28]

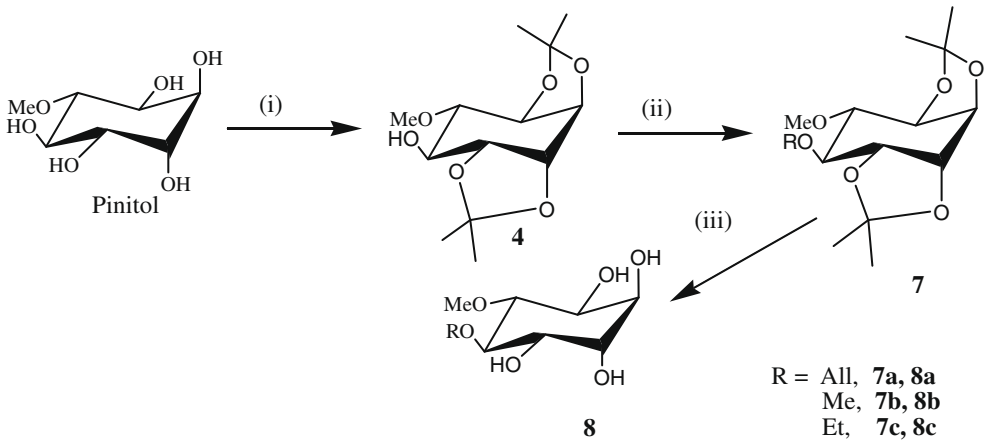
3-Keto derivative **10** was prepared by oxidation of diacetonide, **4** (822 mg, 3.0 mmol) with pyridinium chlorochromate (PCC, 1.290 g, 6.0 mmol) in dichloromethane (DCM) at 0 °C (5 h). On the other hand diacetonide **4** was converted into 3-chloro analog **9** with thionyl chloride in excess of pyridine in 45% yield (Scheme 4).



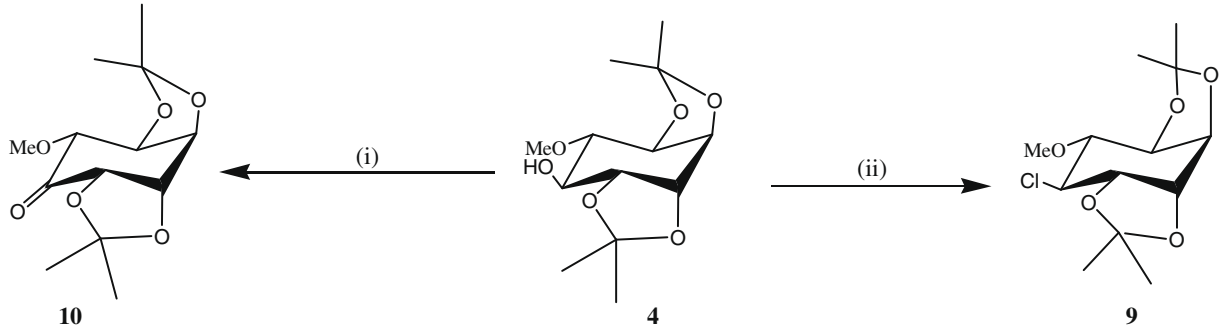
Scheme 1.



Scheme 2. Reagents and conditions: (i) p-TSA, 2,2-dimethoxy propane, dry acetone; (ii) acid anhydride, pyridine; (iii) $HClO_4$–SiO_2/CH_3CN.



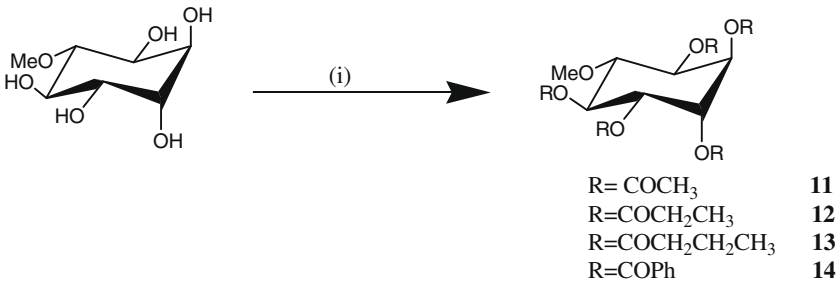
Scheme 3. Reagents and conditions: (i) p-TSA, 2,2-dimethoxy propane, dry acetone; (ii) allyl bromide/methyliodide/ethyliodide, NaH, DMF; (iii) TFA/H_2O (1:1).



Scheme 4. Reagents and conditions: (i) PCC, DCM; (ii) $SOCl_2$, Pyridine.

From ^{1}H NMR studies including coupling constants, it was established that chlorination occurred with the retention of configuration. The J-value of the double doublet for the proton at C-3 position in **4** is almost the same as that of the proton at C-4 position in 1D-3-chloro-1,2:5,6-di-*O*-isopropylidene-4-*O*-methyl-*chiro*-inositol (**9**). Penta-acyl derivatives of pinitol, that is, D-1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-*chiro*-inositol (**11**), 1D-3-*O*-methyl-1,2,4,5,6-penta-*O*-propanoyl-*chiro*-inositol (**12**), D-1,2,4,5,6-penta-*O*-butanoyl-3-*O*-methyl-*chiro*-inositol (**13**), and 1D-1,2,4,5,6-penta-*O*-benzoyl-3-*O*-methyl-*chiro*-inositol (**14**) were prepared from pinitol (5 mmol), and respective alkanoic anhydride (25 mmol) in presence of dimethyl amino pyridine (DMAP, 10–15 mg) by known methods.

Flowcytometric studies were carried out to determine the anti-inflammatory effect of pinitol (Schemes 2–5) and its derivatives on TNF-α cytokine expression in LPS activated human neutrophils. Neutrophils play essential roles in host defence through their ability to clear bacterial infections. Their inappropriate activation contributes to a variety of inflammatory diseases, from the acute respiratory distress syndrome to asthma, chronic obstructive pulmonary disease and rheumatoid arthritis. TNF-α cytokine was chosen as the target for anti-inflammatory activity screening because of the fact that it is a pro-inflammatory cytokine and plays a major role in the pathogenesis of septic shock induced by LPS (Lipopolysaccharide) endotoxin injection.[29] LPS is an important triggering factor for in vivo systemic inflammatory response.[30] LPS activates neutrophils via engagement of TLR4 (Toll-like receptor 4),[31] resulting in the induction of a characteristic proinflammatory phenotype and prolongation of cell lifespan.[32] In brief, neutrophils extraction was performed by taking human blood and centrifuging it at 250*g* for 20 min. Two layers were formed, the platelet rich upper layer was discarded and the lower layer centrifuged at 1800*g*. Again three layers were formed: the platelet poor upper layer was discarded, the middle layer which appeared to be the buffy coat layer



Scheme 5. Reagents and conditions: (i) acid anhydride, pyridine.

was taken and the lower layer which was RBC rich layer was also discarded. Middle layer was taken and layered with histopaque and incubated for 10 min. The samples were centrifuged at 700 g. After centrifugation two layers were observed, upper layer was taken and Facs Lysing solution added for the lysis of any traces of RBC. The samples were washed with PBS (200 g) for 10 min. The intracellular TNF-α level was then estimated in gated population of neutrophils. Neutrophils separation was carried out as described (A new method for detecting TNF-α secreting cells using direct-immunofluoresence surface membrane staining).[33] Cells were stimulated with LPS and incubated with test compounds for 3 h, in CO_2 incubator. For the permeabilisation of the cells Permeablising sol. (1X) (BD-Biosciences) was added and incubated for 10 min. The cells were then labeled with conjugated anti-mouse TNF-α monoclonal antibody (BD-Biosciences). Incubation of the cells was carried out for 30 min in dark. After washing with PBS (Phosphate buffered saline, Sigma Chemicals, USA) the samples were acquired directly on BD-LSR Flowcytometer (BD-LSR, Beckton–Dickinson Biosciences, CA, USA). A fluorescence trigger was set on the PE (FL1) parameter of the gated neutrophil populations (10,000 events). Fluorescence compensation, data analysis, and data presentation was performed using Cell Quest Pro software (Beckton–Dickinson Biosciences, CA, USA).

Most of the semi-synthetic analogs displayed a range of TNF-α expression inhibition from low to high. Pinitol displayed average inhibition of the expression of TNF-α in the range of 30.03% using flowcytometry. From the results given in Table 1, (flowcytometric studies) it is clear that analogs **10** (1D-1,2: 5,6-di-*O*-isopropylidene-3-oxo-4-*O*-methyl-*chiro*-inositol), **13** (1D-1,2,4,5,6-penta-*O*-butanoyl-3-*O*-methyl-*chiro*-inositol), and **14** (1D-1,2,4,5,6-penta-*O*-benzoyl-3-*O*-methyl-*chiro*-inositol) displayed maximum inhibitory effect on TNF-α cytokine secretion in murine isolated neutrophils in response to LPS stimulant. Neutrophils were in vitro treated with 1.0 μg/ml concentration of **10, 13**, and **14** showing 50.57%, 52.47%, and 51.71% TNF-α suppression respectively. The compounds showing more than 50% inhibition of TNF-α expression are considered to have potent TNF-α inhibitory activity. It was found to be efficacious in suppressing intracellular TNF-α level at the dose of 1.0 μg/ml when compared to the LPS control. Therefore, these compounds can be considered as lead molecules for detailed investigations of their anti-inflammatory potential both in vitro and in vivo.

Table 1
Effect of different pinitol derivatives on intracellular TNF-α expression by LPS activated neutrophils

S.No.	Sample	Concn (μg/ml)	Mean ± S.E.	Activity (%)
	LPS Control	1	2.63 ± 0.01	—
1	**Pinitol**	1	1.84 ± 0.04	30.03 ↓
2	**2**	1	2.60 ± 0.08	1.14 ↓
3	**3**	1	1.75 ± 0.04	33.46 ↓
4	**4**	1	2.19 ± 0.11	13.09 ↓
5	**5a**	1	1.51 ± 0.02	42.58 ↓
6	**5b**	1	1.59 ± 0.03	39.54 ↓
7	**5c**	1	1.71 ± 0.02	32.14 ↓
8	**5d**	1	2.19 ± 0.15	20.65 ↓
9	**6a**	1	2.57 ± 0.04	2.28 ↓
10	**6b**	1	2.41 ± 0.08	8.36 ↓
11	**6c**	1	2.08 ± 0.01	20.91 ↓
12	**6d**	1	2.07 ± 0.11	17.85↓
13	**7a**	1	1.71 ± 0.07	34.98 ↓
14	**7b**	1	1.68 ± 0.04	33.33 ↓
15	**7c**	1	1.62 ± 0.04	38.40 ↓
16	**8a**	1	1.85 ± 0.05	29.65 ↓
17	**8b**	1	1.60 ± 0.02	39.16 ↓
18	**8c**	1	1.67 ± 0.02	36.50 ↓
19	**9**	1	1.64 ± 0.05	37.64 ↓
20	**10**	1	1.30 ± 0.03	50.57 ↓
21	**11**	1	1.75 ± 0.04	33.46 ↓
22	**12**	1	1.82 ± 0.09	30.79 ↓
23	**13**	1	1.25 ± 0.02	52.47 ↓
24	**14**	1	1.27 ± 0.06	51.71 ↓
25	**Rolipram**	10	1.24 ± 0.07	52.85 ↓

Samples showing TNF alpha suppression around 50% are considered to be active.
%↓: indicates suppression of TNF- alpha expression; No. of observations = 3.

Partial information about SAR has also been obtained from these studies, though no definite relationship could be established. In general, the molecules protected with ester or acetonide groups displayed much higher TNF-α inhibition than the corresponding unprotected compounds. Furthermore, the dialkylated molecules, for example, **8a–c** exhibited higher activity than their acylated counterparts (e.g., **6a–c**). In case of alkylated analogs, for example, **6a**–**c**, respectively, the bioactivity improved with the increase in the length of side chain; however, the effect is reversed in case of **5a**–**c,** where the activity decreased with increase in the length of side chain. The oxo derivative (**10**) was more active than the chloro derivative (**9**), the reason possibly may be attributed to the presence of oxo functionality which could be involved in the interaction (through hydrogen bonding) at the receptor site. Out of all semi-synthetic analogs, the peracylated derivatives, with longer alkyl chain were found to be more potent than those with smaller alkyl chain. Much higher TNF-α inhibition displayed by compound **3** in comparison to **2** can not be explained though it may be attributed to stereochemical factors (position of hydroxyl groups), given that the molecule is having 6 chiral centers. It appears that lipophilicity played an important role and improved the inhibitory activity, indicating that alkylated part of the molecule probably interacts with the binding site of TNF-α receptor.

In conclusion 23 semi-synthetic analogs of pinitol were prepared using chemo and chemo-enzymatic methodologies. Some interesting structure activity relationships (SAR) could be drawn from the data. However, the three analogs viz. **10**, **13**, and **14** displayed significant TNF-α inhibition activity, which is almost one log higher than the known standard rolipram, and just about double as compared to parent molecule pinitol. The identified lead molecules are being studied to ascertain their potential as chemotherapeutic anti-inflammatory agents.

References and notes

1. (a) Posternak, T. *The Cyclitols*; Holden-Day: San Francisco, CA, 1962; (b) Anderson, L. The Cyclitols, 2nd ed.. In *The Carbohydrates*; Pigman, W., Horton, D., Eds.; Academic: San Diego, CA, 1972; Vol. 1A, p 519.
2. (a)*Inositol Lipids in Cell Signalling*; Michell, R. H., Drummond, A. H., Downes, C. P., Eds.; Academic: San Diego, CA, 1989; (b) Powis, G.; Kozikowski, A. P. Inhibitors of *myo*-Inositol Signalling. In *New Molecular Targets for Cancer Chemotherapy*; Kerr, D. J., Workman, P., Eds.; CRC: Boca Raton, LA, 1994; p 81; (c) Kozikowski, A.; Fauq, A.; Malaska, M.; Tuchmantel, W.; Ognyanov, V.; Powis, G. *Curr. Med. Chem.* **1994**, *1*, 1; (d) Schedler, D. J. A.; Baker, D. C. *Carbohydr. Res.* **2004**, *339*, 1585.
3. For reviews on the synthesis of inositols and conduritols, see: (a) Balci, M.; Sutbeyaz, Y.; Secuen, H. *Tetrahedron* **1990**, *46*, 3715; (b) Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195.
4. Posternak, T. *Helv. Chim. Acta.* **1936**, *19*, 1007.
5. Anderson, A. B.; Mac Donald, D. L.; Fischer, O. L. H. *J. Am. Chem. Soc.* **1952**, *74*, 1479.
6. Naryanan, C. R.; Joshi, D. D.; Mudjumdar, A. M.; Dhekne, V. V. *Curr. Sci.* **1987**, *56*, 139.
7. Anderson, A. B. *Ind. Eng. Chem.* **1953**, *45*, 593.
8. Timotiwu, P. B.; Sakurai, N. *J. Plant Res.* **2002**, *115*, 77.
9. (a) Chaubal, R.; Pawar, V. P.; Hebbalkar, D. G.; Tungikar, B. V.; Puranik, G. V.; Deshpande, H. V.; Deshpande, R. N. *Chem. Biodivers.* **2005**, *2*, 684; (b) Kuo, M. T.; Lowell, A. C.; Nelsen, C. T. *Phytochemistry* **1997**, *45*, 29; (c) Misra, L. N.; Siddiqi, S. A. *Curr. Sci.* **2004**, *87*, 1507; (d) Sherrard, E. C.; Kurth, E. F. *Ind. Eng. Chem.* **1928**, *20*, 722.
10. Fonteles, M. C.; Huang, L. C.; Larner, J. *Diabetologia* **1996**, *39*, 731.
11. Ortmeyer, H. K.; Huang, L. C.; Zhang, L.; Hansen, B. C.; Larner, L. *Endocrinology* **1993**, *132*, 646.
12. (a) Bates, S. H.; Jones, R. B.; Bailey, C. J. *Brit. J. Pharmacol.* **2000**, *130*, 1944; (b) Campbell, W. W.; Haub, M. D.; Flukey, J. D.; Ostlund, R. E.; Thyfault, J. P.; Carrithers, H. M.; Hulver, M. W.; Birge, Z. K. *Hum. Nutr. Metabol.* **2004**, 2998.
13. Iida, K. T.; Suzuki, H.; Kawakami, Y. *J. Biol. Chem.* **2001**, *276*, 32531.

14. Peraldi, P.; Spiegelman, B. *Mol. Cell Biochem.* **1978**, *182*, 169.
15. Falshaw, A.; Hart, J. B.; Tyler, P. C. *Carbohydr. Res.* **2000**, *329*, 301.
16. (a) Dwek, A. R.; Butters, D. T.; Platt, M. F.; Zitzmann, N. *Nat. Rev. Drug Discovery* **2002**, *1*, 65; (b) Ogawa, S.; Fujieda, S.; Sakata, Y.; Ishizaki, M.; Hisamatsu, S.; Okazaki, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3461.
17. Zhan, T.; Lou, H. *Carbohydr. Res.* **2007**, *342*, 865.
18. Chanutin, A. *J. Biol. Chem.* **1926**, *67*, 29.
19. Greenwood, M.; Kreider, R. B.; Rasmussen, C.; Almada, A. L.; Earnest, C. P. *J. Exercise Physiol.* **2001**, *4*, 41.
20. Singh, R. K.; Pandey, B. L.; Tripathi, M.; Pandey, V. B. *Fitoterapia* **2001**, *72*, 168.
21. Kim, J. C.; Shin, J. Y.; Shin, D. H.; Kim, S. H.; Park, S. H.; Park, R. D.; Park, S. C.; Kim, Y. B.; Shin, Y. C. *Phytother. Res.* **2005**, *19*, 1048.
22. (a) Chatterjee, A.; Pakrashi, S. C. *The treatise on Indian medicinal plants*; CSIR: New Delhi, India, 1991; (b) Singh, R. K.; Pandey, B. L. *J. Basic Appl. Biomed.* **1997**, *5*, 25; (c) Singh, R. K.; Pandey, B. L. *Phytother. Res.* **1997**, *11*, 535; (d) Singh, R. K.; Nath, G.; Goel, R. K.; Bhattacharya, S. K. *Ind. J. Exp. Biol.* **1998**, *36*, 187.
23. Choi, M. S.; Lee, W. H.; Kwon, E. Y.; Kang, M. A.; Lee, M. K.; Park, Y. B.; Jeon, S. M. *J. Med. Food* **2007**, *10*, 594.
24. Bauer, J.; Berkenbosch, F.; Van-Dam, A. M.; Dijkstra, C. D. *J. Neuroimmunol.* **1993**, *48*, 13.
25. *Enzymatic Preparation of* D-*1,2,3,5-tetra-O-acetyl-4-O-methyl-chiro-inositol* (**2**) *and* D-*1,2,4,5-tetra-O-acetyl-3-O-methyl-chiro-inositol* (**3**): A suspension of D-1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-*chiro*-inositol (**1**) (808 mg, 2 mmol), CAL (A+B) in powder form (600 mg) and sodium phosphate buffer (0.1 M, pH 7.0, 5 ml) was stirred at 30 ± 1 °C, maintaining pH 7.0 by the addition of 0.01 M NaOH solution. The course of reaction was monitored by TLC. After the completion of reaction the contents were centrifuged at 10,000 rpm. The clear solution and the centrifuged cell pellet were extracted separately with ethylacetate (3 × 25 ml). The organic layers were combined and washed with water, dried and concentrated under reduced pressure. The resulting crude mixture was separated by column chromatography over silica gel to furnish D-1,2,3,5-tetra-*O*-acetyl-4-*O*-methyl-*chiro*-inositol (**2**) (272 mg, 0.75 mmol) as a semi solid and D-1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-*chiro*-inositol (**3**) (250 mg, 0.69 mmol) as a semisolid, respectively.
Compound **2**: IR (KBr): 3479, 2938, 1752, 1372, 1227, 1078, 1046, 983; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.04 (3H, s), 2.13 (3H, s), 2.16 (6H, s), 3.47 (3H, s), 3.56 (1H, m), 3.99 (1H, d, J = 10.0 Hz), 5.16 (2H, m), 5.30 (1H, d, J = 6.2 Hz), 5.35 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.3, 61.4, 67.2, 69.7, 71.6, 72.1, 75.5, 78.5, 168.5,168.8, 170.2, 171.4.; MS m/z (%): M^+-H_2O = 345(4), 289(6), 259(5), 242(11), 213(10), 210(46), 200(42), 188(34), 182(68),169(29), 168(100), 158(45). Anal. Calcd for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found C, 49.83; H, 6.01.
Compound **3**: IR (KBr): 3480, 2939, 1750, 1372, 1232, 1052, 756; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.98 (3H, s), 2.01 (6H, s), 2.07 (3H, s), 3.37 (3H, s), 3.57 (1H, t, J = 10.8 Hz), 4.10 (1H, s), 5.26 (1H, d, J = 9.0 Hz), 5.34 (1H, s), 5.39 (1H, t, J = 9.7 Hz), 5.46 (1H, t, J = 3.9 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 60.3, 68.1, 69.0, 70.0, 70.5, 71.4, 79.2, 168.1, 168.5, 169.2, 170.2; MS: M^+-H_2O = 345(2), 289(8), 259(6), 242(13), 213(14), 210(40), 200(35), 188(37), 182(60), 169(38), 168(100), 158(35). Anal. Calcd for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found C, 49.67; H, 6.23.
26. *Preparation of* 1D-3-*O*-butanoyl-4-*O*-methyl-*chiro*-inositol (**6b**): Freshly prepared $HClO_4$–SiO_2[27] (70 mg) were added to a solution of 1D-3-*O*-butanoyl-1,2: 5,6-di-*O*-isopropylidene-4-*O*-methyl-*chiro*-inositol (1.0 mmol) in acetonitrile (5 ml) and the reaction mixture was stirred at room temperature for 45 min. After the completion of reaction, the reaction mixture was filtered with ethylacetate through Celite bed and concentrated to give the title compound. The sample was further purified by flash chromatography with ethyl acetate:toluene (70:30) as the eluent to give **6b** in 90% yield. Compound **6b**: IR (KBr): 3385, 2965, 2935, 1728, 1383, 1192, 1089, 670; ^{1}H NMR (200 MHz, D_2O): δ 0.98 (3H, d, J = 7.4 Hz), 1.68 (2H, m), 2.38 (2H, t, J = 7.2 Hz), 3.31 (1H, t, J = 1.5 Hz), 3.51 (3H, s), 3.81–3.92 (4H, m), 4.85 (4H, bs), 5.15 (1H, t, J = 10.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0, 18.5, 36.5, 60.0, 69.6, 69.9, 71.7, 71.9, 74.6, 81.2, 170.0; ESI-MS (m/z) = 287 $[M+Na]^+$; Anal. Calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.63. Found C, 50.10; H, 7.51.
27. Agnihotri, G.; Misra, A. K. *Tetrahedron Lett.* **2006**, *47*, 3653.
28. Hart, J. B.; Kroger, L.; Falshaw, A.; Falshaw, R.; Farkas, E.; Thiem, J.; Win, A. L. *Carbohydr. Res.* **2004**, *339*, 1857.
29. Remick, D. G.; Strieter, R. M.; Eskandari, M. K.; Nguyen, D. T.; Genord, M. A.; Raiford, C. L. *Am. J. Path.* **1990**, *136*, 49.
30. Bone, R. C. *Chest* **1991**, *100*, 802.
31. Sabroe, I.; Read, R. C.; Whyte, M. K. B.; Dockrell, D. H.; Vogel, S. N.; Dower, S. K. *J. Immunol.* **2003**, *171*, 1630.
32. Lee, A.; Whyte, M. K. B.; Haslett, C. *J. Leukoc. Biol.* **1993**, *54*, 283.
33. Clara, B.; Arancha, R. C.; Andres, G. M.; Atanasio, P.; Julie, A.; Alberto, O. *J. Immun. Meth.* **2002**, *264*, 77.