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Article

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Medicinal chemistry of dihydropyran based medium ring macrolides related to aspergillides: selective inhibition of PI3Kα[!]

Mallikharjuna R. Lambu, [1,2] *Suresh Kumar,*[1,2] *Syed K. Yousuf,*[2] *Deepak K. Sharma,*[1,2]

Altaf Hussain, [1, 2] *Ajay Kumar,*[2] *Fayaz Malik,*[*1,2] *Debaraj Mukherjee.*[*1,2]

[1]Academy of Scientific and Innovative Research, [2]CSIR-IIIM

Corresponding authors: Tel.: (+) 91-011-256-9000, Fax: (+)-91-011-256-9111. email.dmukherjee@iiim.ac.in, fmalik@iiim.ac.in

Keywords. Macrolides, tri-O-acetyl-D-glucal, ring closing metathesis (RCM), apoptosis, PI3K/AKT.

Abstract: A set of nine trans disubstituted dihydropyran based medium ring macrolides has been synthesised using D- glucal as chiral pool and evaluated against a panel of three human cancer cell lines and a normal cell line. The synthetic route to the targeted molecule is simple, concise and high yielding compare to other reported methods. Bio evaluation studies have resulted in the identification of potent cytotoxic molecule (**10**) exhibiting dose dependent growth inhibition against HL-60 cell line with IC_{50} value of 1.10± 0.75μM which is lower than the naturally occurring molecules of this class and comparable activity to the synthetic drug fludarubin. The compound **10** inhibits PI3K/AKT signaling pathway by selectively targeting p110α subunit of PI3Kα. This leads to mitochondrial stress that causes translocation of cytochrome c from

mitochondria to cytosol, which in turn activate the caspases mediated apoptotic cell death. Further in silico docking simulations of four macrolides with p110α subunits have been carried out to visualize the orientation pattern.

Introduction

Bioactive natural products have had a dramatic impact on medicine and society.[1] They display a seemingly endless structural diversity but are often found only in miniscule quantities. Inspiration from natural products has also brought new perspectives to organic synthesis and chemical biology.[2] The systematic modification of natural products through diverted total synthesis is a powerful concept for the editing of natural products with the aim of studying mechanistic aspects of their biological activity. This concept offers far-reaching opportunities for discovery at the interface of biology and chemistry.[3] Nonetheless, implementation of this type of design require the development of new synthetic strategies in order to modify the structure at will.[4]

Macrocycles represent one of the universal structural features of innumerable number of biologically significant natural products.[5] The structural motifs are highly selective and potent, they are conformationally preorganized, enabling them to bind selectively to targets with minimal entropic loss.[6] In fact these are the smallest examples of bio molecules that exhibit functional subunits. Aspergillides (Figure 1) comprise a novel class of medium ring (14-membered) macrolides.[7] These were isolated from a marine-derived fungus *Aspergillus ostianus* strain 01F313 cultivated in a bromine modified medium[7] and are characterised by the presence of tri-substituted tetrahydro (**1** and **2** in Figure 1) and dihydrofuran (**3** in Figure 1) units.

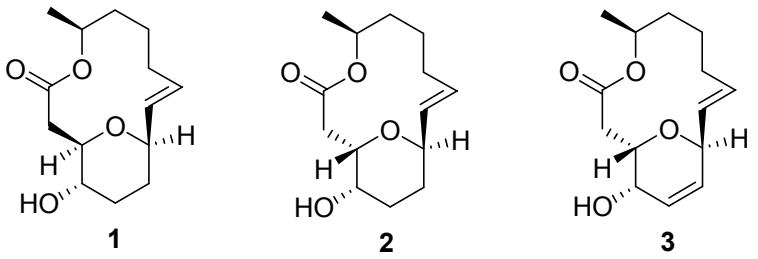

Figure 1. Structures of aspergillides A (**1**), B (**2**) and C (**3**)

These structurally intriguing molecules exhibit cytotoxicity against mouse lymphocytic leukemia cells (L1210) in micromolar range.[8] Owing to their structural features and promising biological properties, aspergillides have always attracted the attention of organic chemists as targets for total synthesis.[8] Although total synthesis of aspergillides A and B are well documented along with limited SAR studies,[9] there are only a few reports of total synthesis of aspergillide C with no structural analogues known.[10] In a programme directed towards the synthesis of medium ring macrocyles,[11] herein we would like to report our study towards the synthesis of a library of medium sized macrocycles fused to a dihydropyran framework and thereafter evaluation of most potent cytotoxic analogs against specific cancer target. Although, the antiproliferative activity of macrolides aspergillide against different cancer cell lines has already been reported, [9c] but the molecular mechanism and the selective cancer driving targeting pathways behind this remain unexplored. It has now become evident that several kinase pathways including phosphatidylinositol 3-kinase (PI3K) and the Raf/mitogen-activated extracellular signal-regulated kinase (ERK) are critical for normal human physiology, and also commonly dysregulated in several human cancers.[12] A large number of studies have shown that the aberrant activation of PI3K/Akt is involved in many human cancers and is responsible for cell proliferation, tumor growth and invasion of cancer cells.[13] It has been suggested that the Inhibition of the PI3K/Akt pathway through small molecules might be a promising strategy for

cancer treatment by inhibiting tumor cell growth and proliferation.[14] An effort has been made to investigate the effect of ring size, stereo diversity and position of double bond of synthesized macrolides on their targeting crucial tumor promoting pathways and antiproliferative activity against different cancer cell lines and to explore the mode of action and of the most active compound. We used in silico approach to check the binding affinities of the compounds towards critical tumor driving p110α subunit of PI3K, which was confirmed through the wet lab experiments with detailed mechanism of action.

Keeping in mind the basic skeleton of aspergillide macrocycles, we proposed two types of structural variants 4 and 5 (Figure 2).

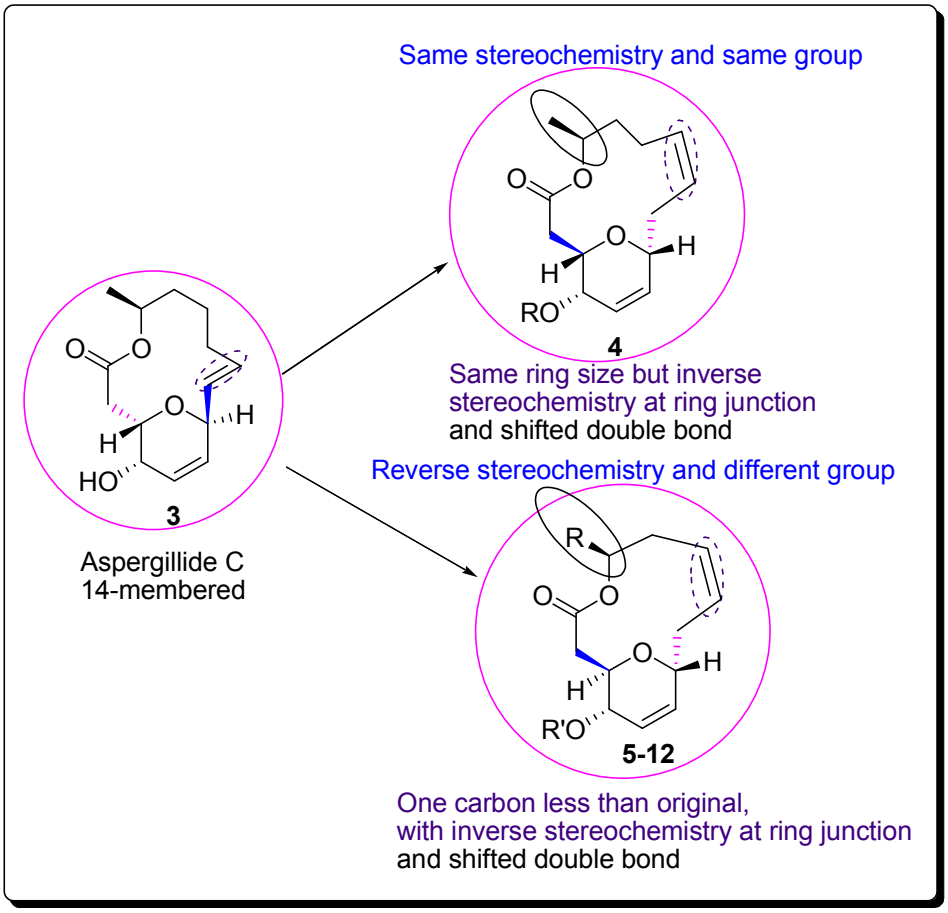

Figure 2. Structures of proposed aspergillide based stereodivergent medium ring macrocycles

The challenges from a synthetic point of view are construction of the bridged 2,6-trans-trisubstituted pyran and cyclisation of the bridged 14-membered macrocyclic core with an olefin at C-8/C-9, which is rare in natural product. It was observed that most reported methods centers on synthesis of 2,6-trans-disubstituted pyran aldehyde/masked aldehyde derivatives (Figure 3), [9b, 10a,10b] which can be obtained from non carbohydrate/carbohydrate precursors followed by olefination of that aldehyde with a chiral alcohol to generate C-8/C-9 double bond and finally macrolactonisation of that alcohol with the *seco*-acid. Although the synthesis of these class of compound has been made, all the methods suffer from longer reaction sequence with low overall yield. Moreover there is no scope of diversifying the macrolides as designed by us in figure 2.

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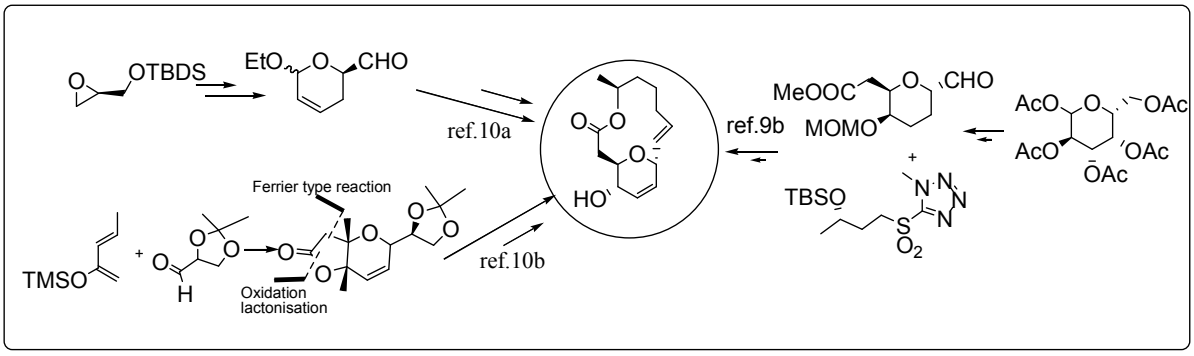
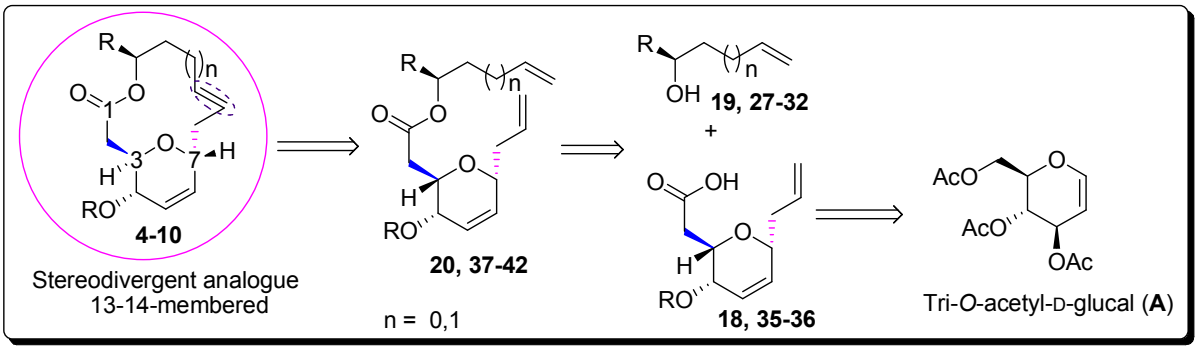


Figure 3. Prior art to the synthesis of 2,6- trans-disubstituted pyran aldehyde derivatives

In order to reduce the number of steps and diversify the core we have chosen D-glucal as chiral pool for our studies considering our previous experience[15] working with it. The synthetic strategy for the medium sized ring macrolides is illustrated in scheme 1.

Scheme 1. Proposed retrosynthetic approach for medium sized ring macrolides

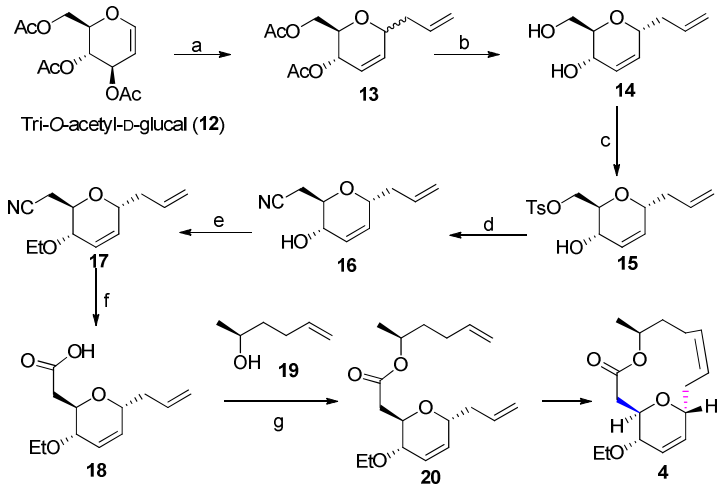


The strategy centres on a Lewis acid mediated stereoselective C-allylation of the readily available tri-*O*-acetyl-D-glucal (A) to fix trans stereochemistry at C-3 and C7 (oppsite stereochemistry relative to parent molecule). The macrocycles could be assembled by using a sequential EDC coupling of a alcohols of appropriate size (**19, 27-32)** with the corresponding sugar derived acids (**18, 35-36**) and ring closing metathesis of the di-olefinic compounds (**20, 37-42)**.

Results and discussions:

Chemistry. In order to achieve trans stereochemistry at ring juncture of pyran ring we started our investigation with α-C-allylation of tri-*O*-acetyl-D-glucal **(A)**. As anticipated α-allyl glycoside **(13)** was obtained in good yield with high stereoselectivity (α:β, 95:5). Subsequent regioselective protection of primary hydroxyl by tosyl group afforded **15**, nucleophilic substitution of –OTs compound with NaCN provided cyano derivative **16**.

Scheme 2. Synthesis of 14-membered macrolide **4**[a]

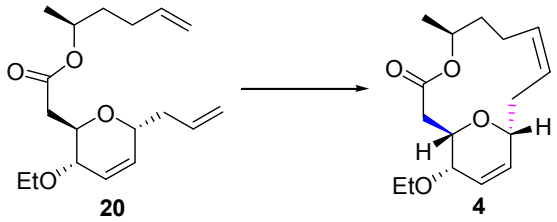


[a]Reagents and conditions: (a) Allyl trimethylsilane (1.2 equiv .), $BF_3.Et_2O$ (1.2 equiv .) -10 ^oC, 3h, 89%; (b) NaOMe, MeOH, rt, 0.5h, 98%; (c) Tosyl chloride (2.5 equiv .) dry pyridine, 0 ^oC-rt, 8h, 65%; (d) NaCN (2.0 equiv .), DMSO, 70 ^oC, 4h, 85%; (e) Ethyl iodide (1.2 equiv .), NaH (1.5 equiv.), 0 ^oC, 2h, 90%; (f) 8*N* NaOH + EtOH (1:1), reflux, overnight, 75%; (g) EDC (1.2 equiv .), DMAP (0.1 equiv .), DCM, rt, 8h, 78%.

After protection of free hydroxyl group as ethyl ether, compound **17** was subjected to cyanide hydrolysis to obtain the key carboxylic acid derivative (**18**) required for esterification. However, hydrolysis of **17** failed to proceed smoothly and caused degradation of starting material. Careful optimization of hydrolysis condition (see SI table1) eventually afforded trans 2,6 disubstituted DHP fragment **18** in satisfactory yield. EDC mediated esterification of acid **18** with optically pure alcohol **19** generated diolefinic compound **20** ready for final macrolide synthesis. Ring closing metathesis (RCM) of **20** in presence of 10 mol % of Grubb's II catalyst at 40 ^oC failed to yield the desired cyclisation product. Nonetheless, after rigorous optimisation of reaction conditions, it was found that the concentration of the substrate plays a critical role in the RCM step (**Table 1**). It is noteworthy that 3 mM DCM solution of **20** gave the desired RCM product **4** with 68% yield. The structure of the product **4** was determined through spectroscopic analysis. The disappearance of peaks between 4.98-5.12 present in the ^1H NMR spectrum of **20** for two

terminal alkene –CH_2's and appearance of new peaks between 5.50-5.34 with J = 11.2 Hz (along with peaks at 126.5 and 125.1 in ^{13}C NMR for the newly generated alkene of **4** indicated the formation of macrolide **4**. Small coupling constant (always <12 Hz) clearly indicated the preponderance of Z-isomer. The ratio of E/Z isomers was deducible from the ^{1}H NMR spectrum.

Table 1. Standardisation of reaction conditions for RCM reaction



Entry	Catalyst[a] (mol %)	Solvent (Molar conc. of substrate)	Temp. (^{0}C)	Yield %[b] (Z/E)[c]
1	10	DCM (15)	40	Nil
2	5	DCM (15)	40	Nil
3	1	DCM (15)	40	6 (50:50)
4	5	DCM (10)	40	16 (50:50)
5	5	DCM (5)	40	20 (60:40)
6	5	DCM (3)	40	35 (75:25)
7	5	DCM (3)	30	42 (85:15)
8	5	DCM (3)	20	55 (90:10)
9	5	DCM (3)	0	38 (99:1)
10	**5**	**DCM (3)**	**0-20**	**68 (99:1)**
11	5	Toluene (15)	40	Nil

[a]In all cases Grubbs II generation catalyst was used. [b]Isolated yield after column chromatography. [c]Determined from 1H NMR .

Encouraged by the ease in accomplishing the stereodivergent synthesis of 14-membered macrolide **4**, an aspergillide C analogue, we next directed our attention towards the synthesis of a set of medium ring macrolides for SAR. Thus, using the same sequence of reactions but by varying the alcohol partner, which was generated from literature procedure.[16] It is noteworthy that changing the protection from methoxy or ethoxy to PMB (**35**) leads to increased yield of acid during cyanide hydrolysis. We generated the analogues of **4** with one carbon less (Figure 4). The compound **5** shows the small coupling constant (always <12 Hz) clearly indicated the preponderance of Z-isomer, the stereo chemistry at C-12 position of the macrolide **5** confirmed by the NOSEY correlation between H-12 and H-3. This confirms proposed structures of the 13 membered macrolides.

Compounds **11** and **12** were obtained in quantitative yield (>90) by DDQ mediated removal of the PMB-protecting group of **7** and **9** respectively

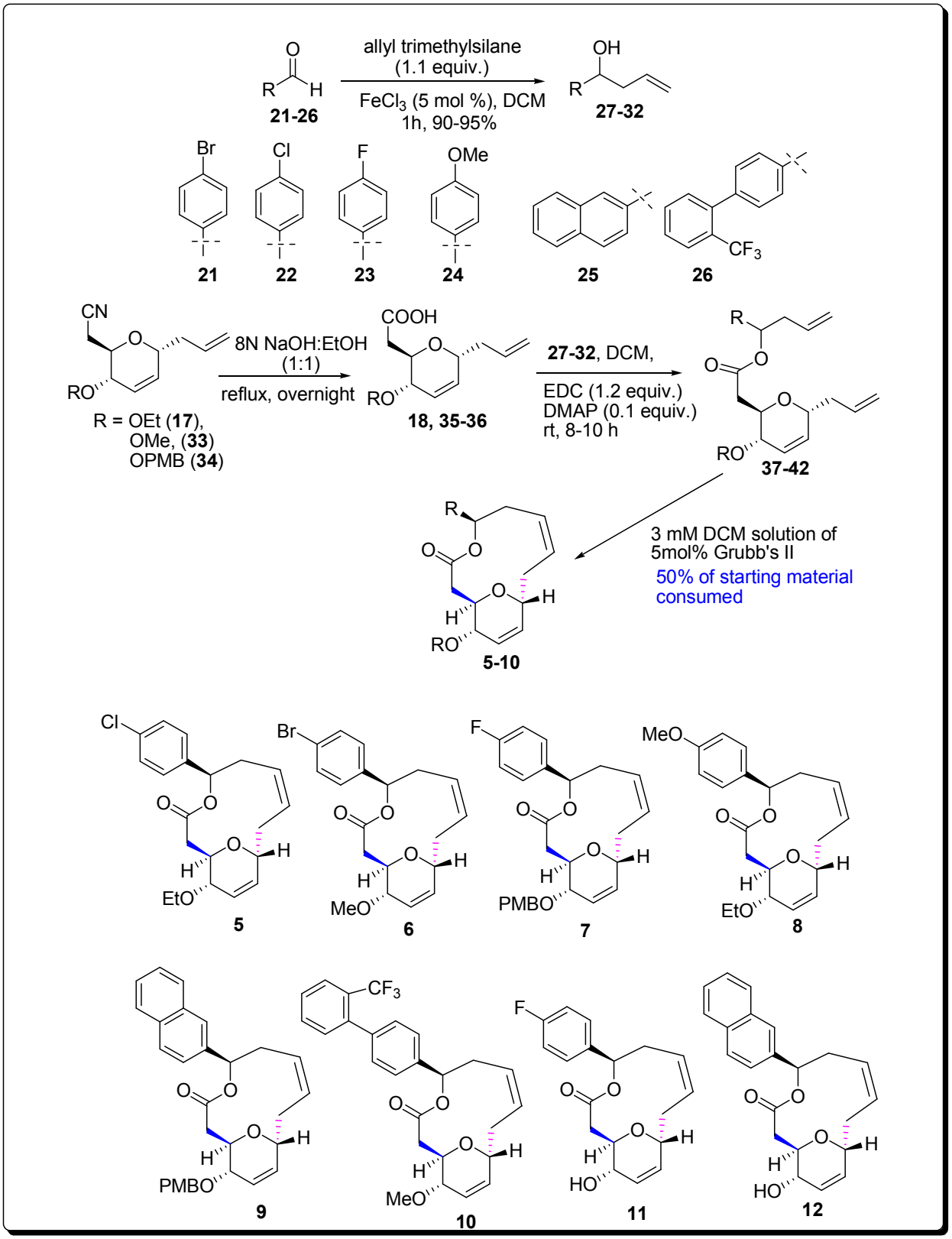


Figure 4. Library of medium sized pyran based macrocycles

Molecular modeling study:

Docking simulations were performed to predict the binding modes of molecules with target protein P110α subunit of PI3K. Docking studies reveals that compounds **10** and **5** having alkoxy protection (OMe, OEt respectively) interacts with Gln-859 and Tyr-836 residues respectively via hydrogen bonding at the catalytic site of the p110α (Figure 5). In addition, hydrophobic interactions between Trp-780, Ile-932 and Met-922 and aromatic rings of the dihydro-pyran based macrocycles are driving force for P110 α binding motifs (Figure 5). However, compound **12** (having hydroxyl group at C-4) adopts different binding pattern and orients toward the Ser-854 residue (Figure 5). Similarly the compound **9** which bears O-PMB functionality at C-4 adopts complete opposite orientation by 180^{o} (Figure 5) thus allowing it to bind with Val-850 of hinge region instead of Gln-859 or Tyr-836 residues of P110α kinase domain.

In order to predict the most suitable analog which can be an inhibitor of P110α, we calculated the prime ligand binding energies (ΔG) of different synthesized analogues by end point MMGB/SA method and compared with known PI3K inhibitors. ΔG value of the compound **10** (-73.96 Kcal/mol) docked with P110α was found to be comparable with known PI3K inhibitor Liphagal (-77.27 Kcal/mol). Compound 10 showed lower binding affinity towards P110β (-58.05 Kcal/mol) and P110δ (-49.78 Kcal/mol) which was further confirmed by docking simulation study (Section D in SI).

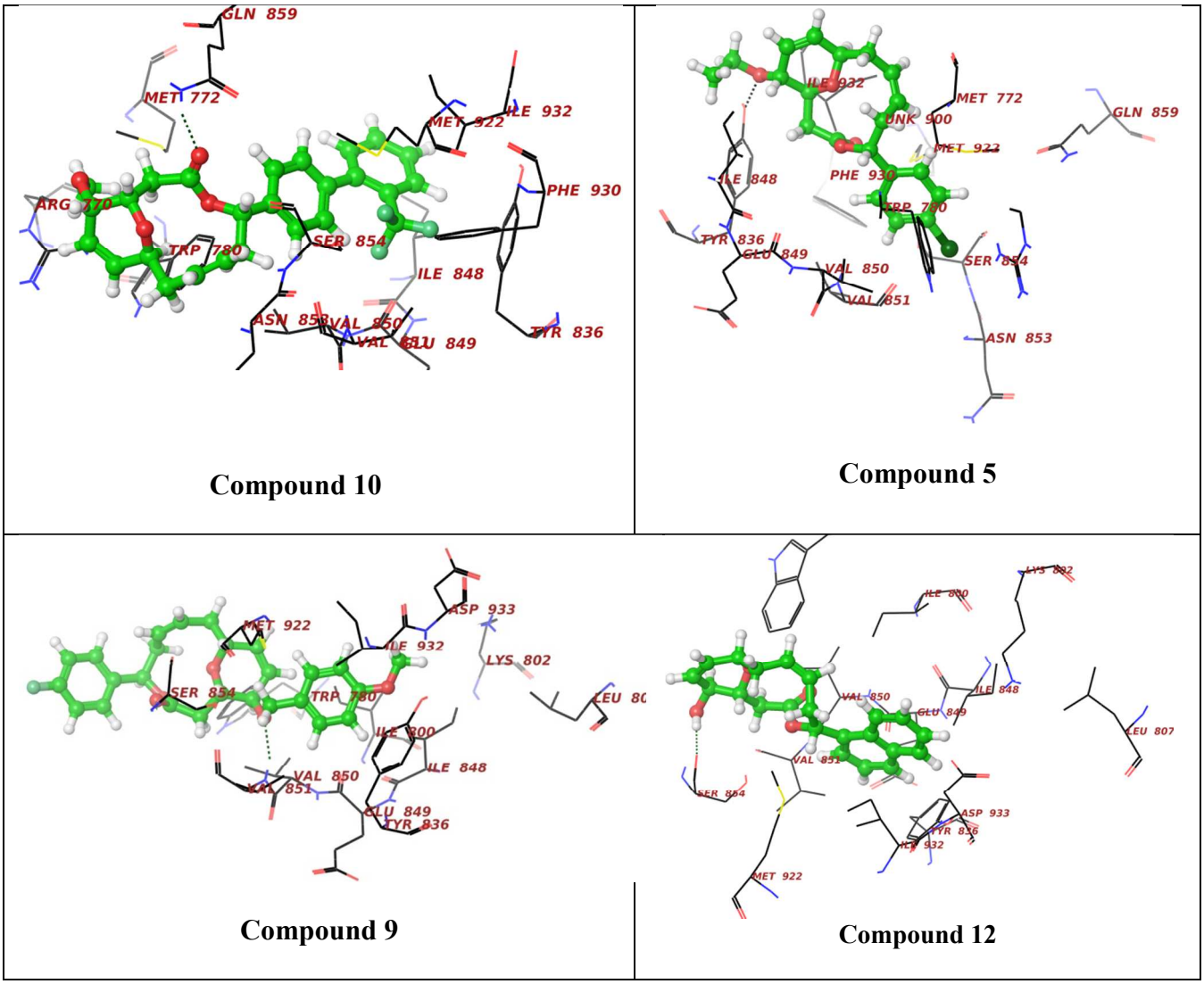


Figure 5. Docking simulations of the compounds **10**, **5**, **9**, and **12** with target protein PI3K-α

Biology. All the newly synthesised macrolides were assayed against various cancer cell lines including HL-60 (acute promyelocytic leukaemia), PC-3 (human prostate cancer cell line), and A375 (human amelanotic melanoma cell line) for their antiproliferative activity. It was found that all compounds exhibited antiproliferative effects at micromolar concentrations (Table 2).

Table 2. Growth inhibitory activity of the macrocycles against various cancer cell lines

S. No.	Compound	IC $_{50}$ in HL-60(μM)	IC_{50} in PC-3(μM)	IC_{50} in A375(μM)	IC_{50} in FR2 (μM)

1	**4**	69.74± 1.54	78± 0.76	57± 2.67	ND
2	**5**	5.7± 0.863	9.16± 0.63	4.9± 0.12	ND
3	**6**	10.00± 0.73	15.76± 1.82	5.45± 2.76	ND
4	**7**	8.83± 0.87	2.23± 0.96	4.76± 1.87	ND
5	**8**	69.9+ 0.92	56.00+ 0.45	62.7± 1.12	ND
6	**9**	10.23± 0.24	45.4± 1.34	>100	ND
7	**10**	1.10± 0.75	7.36± 00.37	9.64± 0.35	≥ 100
8	**11**	40.23± 2.13	35.80± 3.23	20.76± 1.34	ND
9	**12**	30.92± 0.873	21.5± 1.23	50.00± 0.54	ND
10	**1**[9c]	81.2± 17.5	ND	ND	ND
11	**2**[9c]	32.8± 7.6	ND	ND	ND

The IC_{50} values, defined as the drug concentration required for inhibiting cell proliferation by 50%, were calculated from the curve of concentration dependent survival percentage, itself defined as absorbance in experimental wells compared with absorbance in control wells after subtraction of the blank values. It was found that compound **10** is most active among others and we concluded that HL-60 cell line is more sensitive than PC-3 and A375 for the compound **10**. While compound 10 shows potential cytotoxicty against cancer cell lines, we also tried to

evaluate its selective killing by observing it's effect on Human normal breast epithelial cell line "FR2". It was observed that compound 10 confer selective cytotoxicity towards cancer cells while as its IC_{50} value in FR2 cells was not attained till 100 μM concentration. In general, electron withdrawing substituents in the aromatic moiety located at C-12 position lead to the enhanced antiproliferative activity with CF_3 group in compound **10** as most potent followed by halogen substituents like F, Cl and Br in the compounds **7**, **5**, **6** respectively. Presence of a free hydroxyl at C-4 decreases the activity, but smaller alkoxy substituents (Me > Et > PMB) lead to more activity as found in **10**. It is more like lipophilic driven SAR. In other word, potency may gradually increase when the lipophilicity increases. Introduction of biphenyl group increases lipophlicity significantly in compound **10** (clogP = 4.65) and thus improves potency. Similarly, in compound **11**, introduction of a polar OH motif with one hydrogen donor lowers lipophilicity (clogP = 1.87) and hence potency decreases compared to compound **9** (clogP = 4.82). In order to get some mechanistic insight of the mode of action of compound **10** in HL60 cell lines, few more experiments such as phase contrast microscopy, Hoechst staining for nuclear morphology, Mitochondrial membrane potential loss, Annexine V/PI staining, Cell cycle phase distribution and western blotting analysis have been carried out.

Phase contrast microscopy

Phase contrast microscopy was done to observe the morphological changes. Significant changes in morphology of cell were observed. The changes increased with increasing dose of compound 10. Treatment with 5 μM of compound **10** showed blebbing and shrinkage of cells (Figure 6); the amount of apoptosis (blebbing and shrinkage) increased significantly in cells treated with 20 μM of compound 10 (Figure 6).

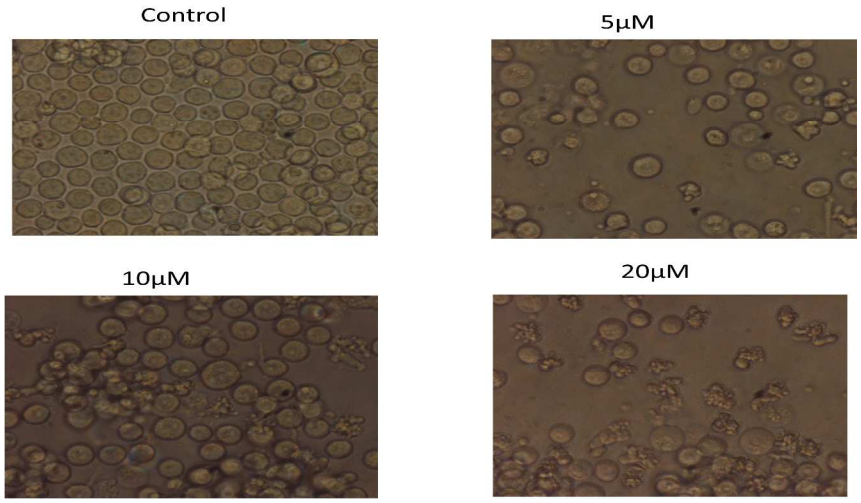


Figure 6. Compound **10** induced cell death in HL-60. Cells were treated with indicated concentrations of compound **10** and observed for morphological changes under microscope (1 × 81, Olympus).

Hoechst staining for nuclear morphology

HL-60 cells treated with different concentrations of compound **10** displayed pronounced changes in nuclear morphology; the change in morphology of nucleus increased with increasing concentrations (Figure 7).

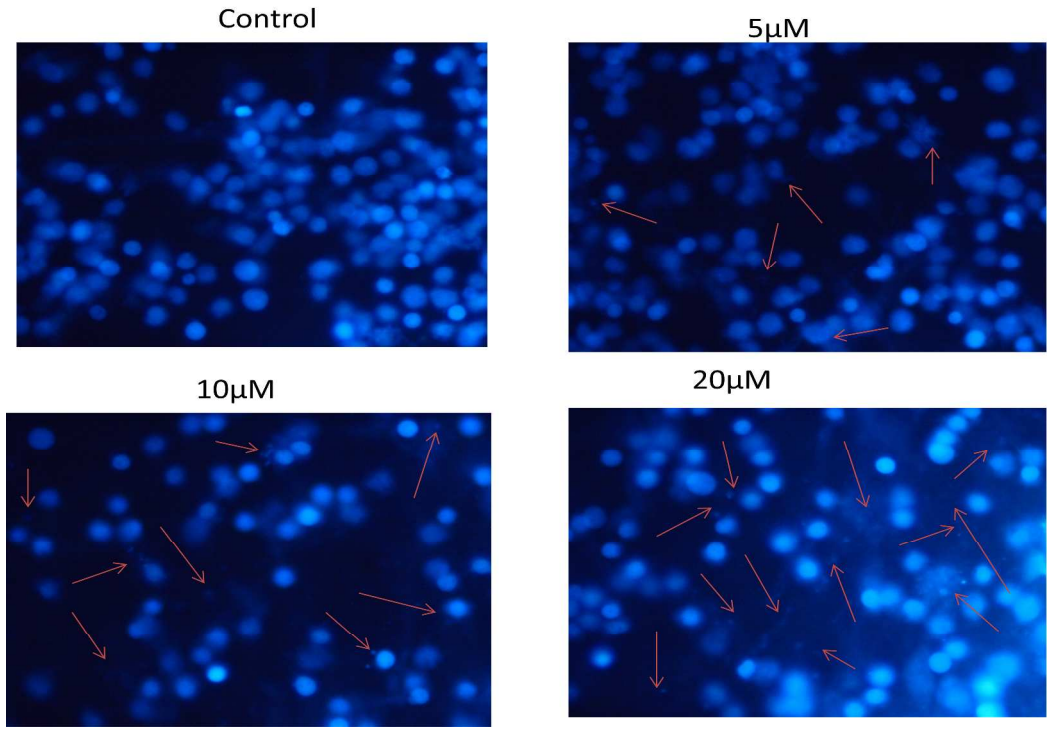
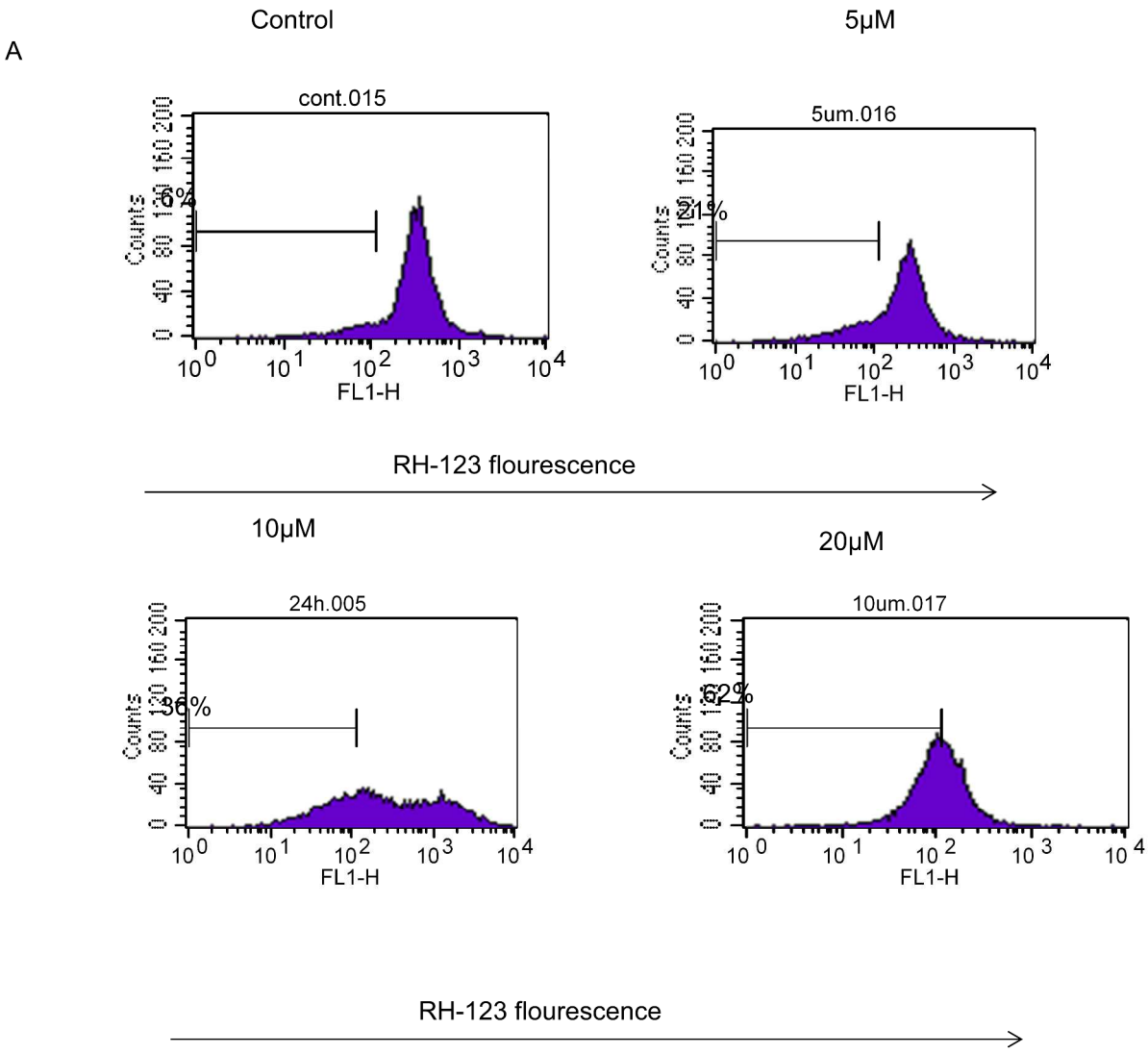


Figure 7. Alteration in nuclear morphology by compound **10** treatment. HL-60 cells were incubated with different concentrations of compound **10**, collected at 400 g, washed once with PBS and then stained with Hoechst 33258 for 30 min. The procedure is discussed in materials and methods section.

Mitochondrial membrane potential loss

Mitochondrial integrity is required for cell to be functional and mitochondrial potential loss is considered as one of the major causes of cell death. Compound **10** induced MMP loss which increased with increasing doses. The loss was significant at 5 μM treatment and further increased to 62% when the cells were treated with 20 μM of compound **10** (Figure 8A). This demonstrates the apoptotic potential of the compound. To ascertain the role of mitochondrial potential in cell death induced by compound **10**, we checked the translocation of cytochrome c from mitochondria to cytosol. A marked decrease in the expression of cytochrome c in mitochondrial fraction with simultaneous increase in cytosolic fraction was observed during the period of

treatment, showing the role of mitochondrial potential loss in apoptosis induced by compound **10** in HL-60 cells (Figure 8B).



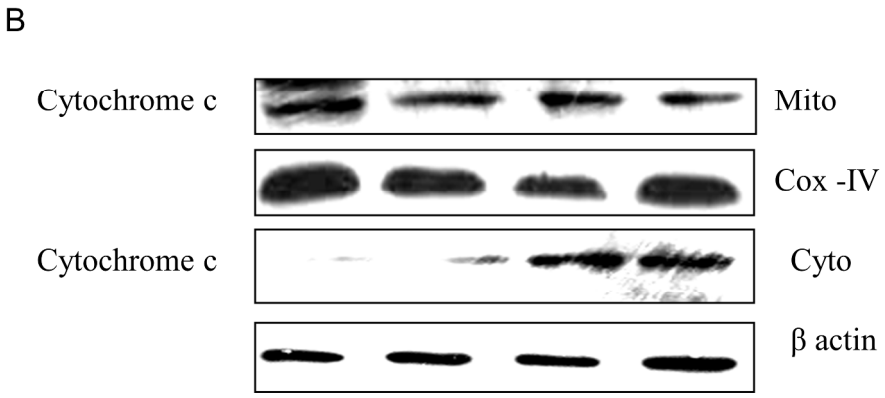


Figure 8. **A.** Compound **10** induced time dependent MMP loss in HL-60 cells. HL-60 cells ($.5\times10^6$) were treated for 6 h with indicated concentrations of compound **10**, washed once with PBS, and stained with RH 123. MMP was measured as discussed in materials and methods section. **B.** compound **10** mediated cytochrome c release from mitochondria to cytosol. 4×10^6 HL-60 cells were treated with indicated concentrations of compound **10** for 6 h. Cell pellet was washed once with PBS and cytosolic and mitochondrial proteins were isolated as described in materials and methods. Translocation of cytochrome c from mitochondria to cytosol was seen through western blot. COX IV was used as internal control for mitochondrial fraction while β actin was used as an internal control for cytosolic frcation.

Annexine V/PI staining

Annexin V/PI staining was performed to distinguish apoptotic and necrotic cell deaths. Cell death induced by compound **10** was purely apoptotic as the amount of necrotic cells (PI stained) was negligible as compared to apoptotic population (Annexin V-FITC stained) of cells. About 25% cells were purely apoptotic after treatment with 5 μM of compound **10** (Figure 9). The apoptosis further increased with increasing doses of compound.

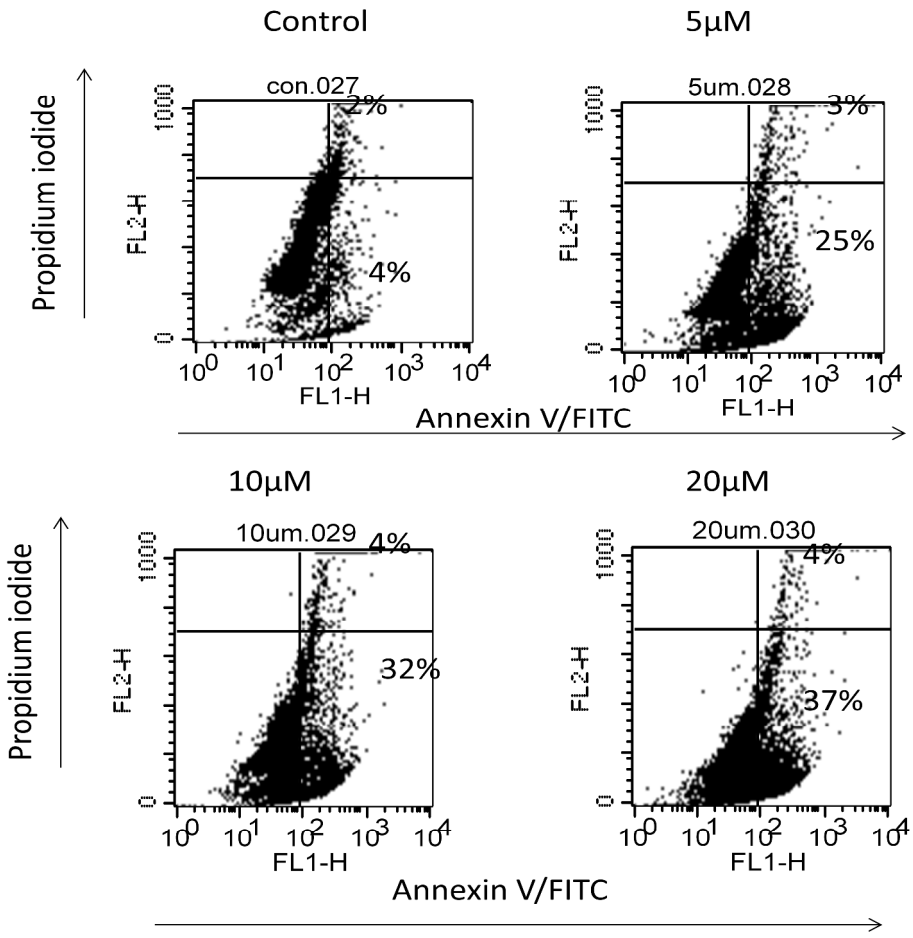


Figure 9. Cell death induced by compound **10** is apoptotic. Treated and untreated HL-60 cells were collected washed once with PBS and stained with annexine V-FITC/PI as explained in materials and methods section.

Cell cycle phase distribution

HL-60 cells treated with compound **10** for 6 h showed considerable increase in apoptotic population (sub G0/G1), the apoptotic population increased from 5% in control sample to 37% in sample treated with 20 μM of compound **10** (Figure 10).

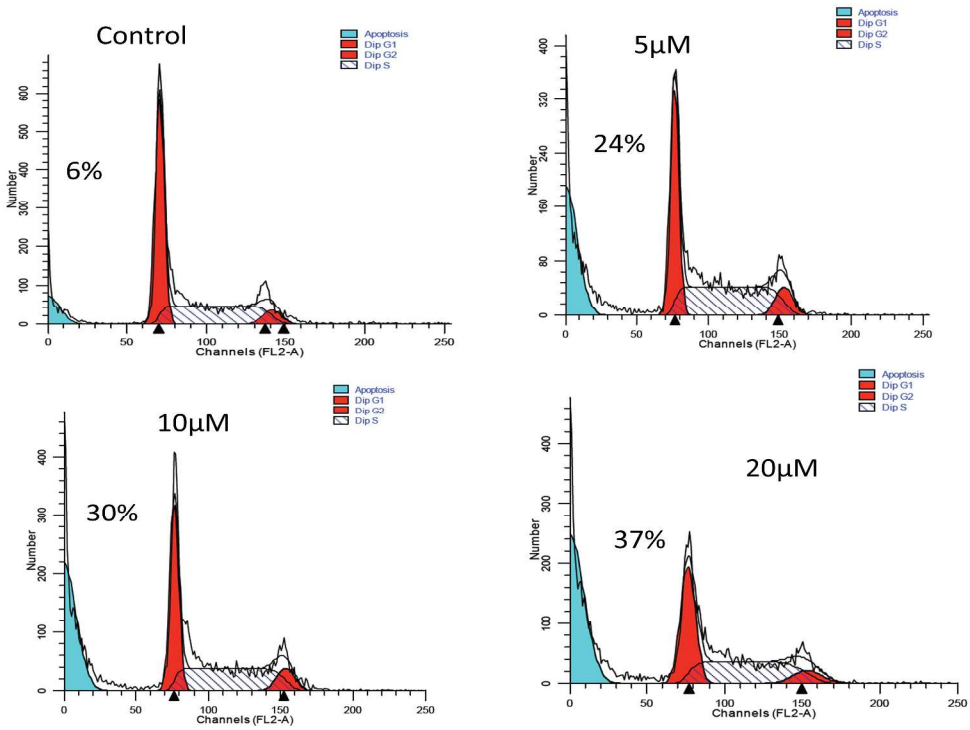


Figure 10. Induction of G0/G1 population. HL-60 cells (1×10^6) were seeded in 12 well plates, treated with different concentrations of compound **10** (5 µM, 10 µM and 20 µM) for 6 h. After completion of time cells were collected at 400 g, washed once with PBS, and fixed in 70% ethanol overnight. Cells were then washed once with PBS and stained with 100 µg of PI for 30 min. Modfit software was used to differentiate between different phases and determine the amount of apoptotic population.

Compound 10 induced apoptosis through the inhibition of PI3K/AKT pathway

Western blot analysis showed that compound **10** inhibited major proteins of PI3K/AKT signaling pathway. The compound inhibited the p110α subunit of PI3K. Interestingly, P110β and P110δ subunits of PI3K were not affected by compound **10** treatments. However, the expression of the major downstream effectors of the pathway was diminished significantly. The effect was more pronounced in both the phosphorylated forms of AKT (T 308) and pAKT(S 473). Also GSK3β, substrate of AKT, was inhibited significantly, but total AKT was not affected.

Furthermore, mTOR a downstream effectors of AKT and its substrate pP70s6k were also inhibited in compound **10** treated cells (Figure 11A). Expression of ERK1/2 was also checked to see the effect of the compound on this pathway. However, the compound did not affect the expression of ERK. Inhibition of the PI3K/AKT signaling has been reported to activate caspase 3[20], which is considered as sign of apoptosis. Compound **10** causes caspase 3 cleavage in HL-60 cells (Figure 11B), which was increased treatment dose. Compound **10** treatment was also found cause PARP-1 cleavage, which play significant role in apoptosis along with caspase 3. Hence, it was found that compound **10** mediated inhibiton of P13K signaling pathway stimulate caspase dependent cancer cell death.

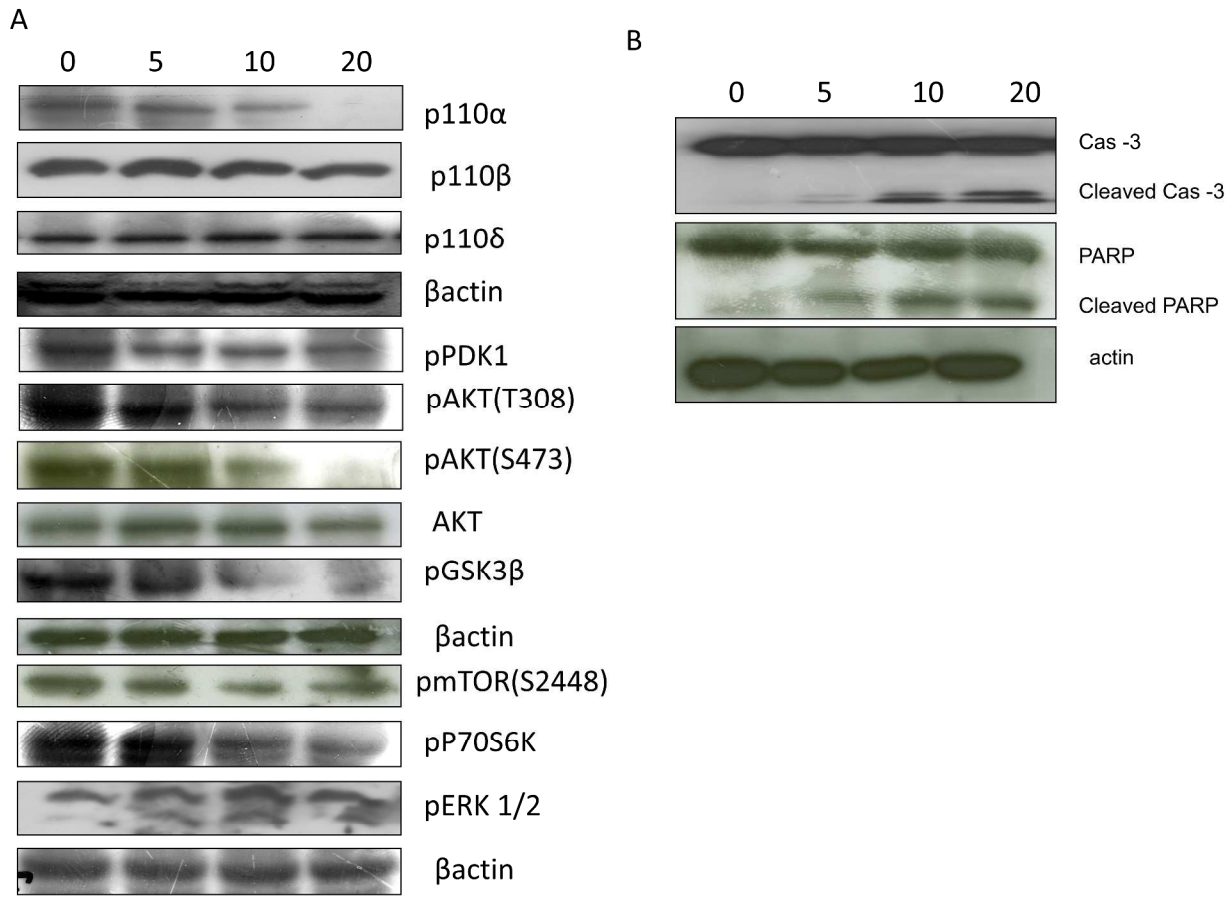


Figure 11. Compound **10** induces apoptosis by inhibiting PI3K/AKT pathway specifically thorough the inhibition of p110α subunit of PI3K. A) HL-60 cells (2×10^6) were treated with

indicated concentrations of compound **10**. Cells were lysed and western blot for different proteins was performed as described in materials and methods. Compound **10** was found to inhibit whole of the PI3K/AKT pathway. β actin was used as internal control. B). Compound **10** induces apoptosis. Treated and untreated cells were collected and lysed in RIPA buffer as described earlier, western blot for PARP-1 and caspase 3 was done, and significant cleavage of PARP-1 and caspase 3 was observed confirming the role of compound **10** in apoptosis. β actin was used as internal control.

Discussions

The present study describes the mechanism of cell death induced by compound **10** in HL-60 cells. Apoptotic potential of the compound was observed through phase contrast microscopy and Hoechst staining, which was further confirmed by annexine V-PI staining. Mitochondrial membrane potential loss has been considered as a mark of cell death.[17] As compound **10** induced mitochondrial membrane potential significantly, and the treated cells were exhibiting G0/G1 arrest of cell cycle, this points to the apoptotic potential of the compound. The PI3K/AKT pathway has been found to play a crucial role in cell proliferation, [18] cell survival, [19] and inhibition of apoptosis.[18] Compound **10** inhibited the major proteins of this pathway. AKT pathway has been found to control the activation of caspase-3, inhibition of AKT being associated with activation of caspase-3.[20] PI3K inhibitor LY294002 has been reported to mediate the translocation of cytochrome c from mitochondria to cytosol,[21] indicating the role of PI3K pathway in cytochrome c release and apoptosis. Similarly, it was found that compound 10 also mediate the release of cytochrome c from mitochondria to cytosol during the cell death induced by compound 10. Since cytochrome c play a role role in activation of caspase 3, [22] it was found that treatment of compound 10 was able to activate the caspase3 followed by PARP1 cleavage.

As compound 10 selectively inhibited P110α subunit of the PI3K, expressions of P110β and P110δ were not affected. So we concluded that compound 10 induce apoptosis by targeting p110 α subunit of the PI3K through mitochondrial stress.

Conclusion

In summary, starting from tri-*O*-acetyl-D-glucal, a set of medium sized macrocycles based on the structural framework of aspergillide has been synthesised. Short, high yielding synthetic steps for targeted molecules with easy processing are some of the advantages of the process. Further, in an effort to investigate the effect of ring size, stereo diversity and position of double bond of synthesized macrolides on their antiproliferative activity against different cancer cell lines, it has been found that one of the derivatives (compound **10**) selectively inhibits tumorigenic PI3K-α/AKT pathway and further modification in the structure of this molecules can be useful to develop new anticancer drugs.

Materials and methods

Biology. RPMI-1640, propidiumiodide (PI), 3-(4,5, -dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Rhodamine 123, penicillin, streptomycin, L-glutamine, Hoechst-33258, pyruvic acid, camptothecin, ribonuclease A, protease inhibitor cocktail were purchased from Sigma chemical Co. Fetal bovine serum was obtained from GIBCO Invitrogen Corporation USA. Tween 20, AnnexinV-FITC apoptosis detection kit, PARP-1, β actin, caspase-3 were purchased from Santa Cruz biotechnology. AKT, pAKT (s473), pAKT , T308 , PII0α (s4249), P70s6k, pmTOR,pGSK3β were acquired from Cell Signaling Technology.

Cell culture, growth conditions and treatment. Human promyelocytic leukemia cells HL-60, were obtained from National Cancer Institute (NCI), Bethesda, USA. The cells were grown in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS),

penicillin (100 units/ml), streptomycin (100 lg/ml), L-glutamine (0.3 mg/ml), pyruvic acid (0.11 mg/ml), and 0.37% $NaHCO_3$. Cells were grown in CO_2 incubator (Thermocon Electron Corporation, USA) at 37^oC in an atmosphere of 95% air and 5% CO_2 with 98% humidity. Different derivatives of macrolides (**4-12**) series were dissolved in DMSO (dimethylsulfoxide) and delivered to cell culture in complete medium.

Cell proliferation assay. Cells were plated in 96 well plates at the density of 15000 cells per well/200µl of the medium. Culture were treated with different concentrations of different molecules of macrolide for 48 hours, 20µl of MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) (2.5mg/ml) was added to each well and incubated at 37^o C for two hours. The plates were centrifuged at 2000 rpm for 15 minutes and supernatant were discarded and the MTT formazen crystals were dissolved in 150µl of DMSO. Plates were shaken on shaker for 3 minutes and then incubated at 37^{o}C for five minutes. The OD measured at 570nm. OD of control samples was equilibrated to 1 and the cell viability of control was considered to be 100%, and accordingly, viability of other samples was calculated by using the following formula.

$$\%\ cell\ viability = \frac{\text{OD (Test)}}{\text{OD (Control)}} \times 100$$

Hoechst 33258 staining of cells for nuclear morphology: HL-60 cells were treated with indicated concentrations of compound **10** for 6 h. Cells were centrifuged at 400 g for 5min and washed twice with PBS. Cells were then stained with one milliliter of staining solution (Hoechst 33258, 10 µg/ml of 0.01M citric acid and 0.45 M disodium phosphate containing 0.05% Tween 20) and stained for 30 min under subdued light at room temperature. After staining the cells were resuspended in 50 µl of mounting fluid (PBS:glycerol, 1/1), 10 µl mounting solution, containing cells was spread on clean glass slides and covered with the cover slips. The slides were then

observed for any nuclear morphological alterations and apoptotic bodies under inverted fluorescence microscope (Olympus 1X70, magnification 30X) using UV excitation.

Cell cycle analysis. Effect of compound **10** on different phases of cell cycle was assessed by propidium iodide fluorescence. HL-60 cells 1×10^6 per well, were incubated with different concentrations of compound **10** (5,10,20 μM) for 6 h. The cells were then washed twice with ice-cold PBS, harvested, fixed with ice-cold PBS in 70% ethanol and stored at -20^{o}C overnight. After fixation, these cells were incubated with RNAse A (0.1 mg/mL) at 37^{o}C for 90 min, stained with propidium iodide (100 μg/mL) for 30 min on ice in dark, and then measured for DNA content using BD FACS flow cytometer (Becton Dickinson, USA). Data were collected in list mode and 10,000 events were analysed for FL2-A vs. FL2-W. Modfit software was used to distinguish different phases of cell cycle.

Measurment of mitochondrial membrane potential. For mitochondrial membrane potential 1×10^6 HL-60 cells were seeded in 12 well plate and incubated with compound **10** (5 μM,10 μM and 20 μM) for 6 h. RH123(200 nM/ml) was added 30 minutes before termination of experiment. Cells were collected at 400g, washed once with PBS and mitochondrial membrane potential was measured in FL-1 channel of flow cytometer.

Annexin /PI staining to measure apoptosis and necrosis simultaneously. 1×10^6 HL-60 cells were treated with 5 μM ,10 μM and 20 μM of compound **10** for 6 h. Cells were double stained with annexin V/PI by using kit manufacture's protocol (santa cruze biotechnology, sc4252). The FACS analysis for apoptosis and necrosis was done as described earlier.[23]

Phase contrast microscopy. Phase contrast microscopy was done to assess the morphological changes on cells after treatment with compound **10**. Cells were incubated in six well plates and treated with different concentration of compound **10** for 6 h, after completion of time cells were

subjected to photography. Apoptosis was assessed by using microscope (1X70, Olympus), photographs were taken by using DP-12 camera.

Western blot analysis. Treated and untreated HL-60 cells were centrifuged at 400g at 4^{o}C, washed in PBS and cell pellets were lysed in RIPA buffer for preparation of whole cell lysate as described earlier.[24] Equal amount of protein (60 μg) was loaded into each well for SDS-PAGE. Blots were incubated with different primary antibodies, and chemiluminiscence was captured on hyperfilm after incubating the blots in ECL plus solution.

Preparation of mitochondrial and cytoslic lysates. 4×10^{6} HL-60 cells were seeded in 90mm tissue culture dishes and treated with indicated concentrations of compound 10 for 6 h. cytocolic fractions were isolated by incubating cells with lysis buffer containing digitonin for 2 mins and centrifuging the lysates at 12000g, while mitochondrial fractions were lysed with RIPA buffer as described early.[24]

Experimental section

Molecular modeling study

The docking studies of molecules were performed using the Schrodinger software suite (Maestro, version 9.2).[25] The macrocyclic compound **10** were sketched in 3D format using build panel and were prepared for docking using LigPrep application. The Protein coordinates of PI3K-alpha (PDB ID: 2RD0) for docking study were taken from protein data bank (PDB).[26] Protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS2005 force field.[27] Grids for molecular docking were generated with wortmannin (from PDB ID 3HHM).[28] Wortamanin coordinates were adopted to 2RD0 and were used as the ligand to generate the grid for the docking study. Compounds were docked using Glide module, [25] with up to three poses saved per molecule.

Chemistry. All the reagents used were purchased from sigma Aldrich. Solvents were distilled before use. ^{1}H and ^{13}C NMR spectra were recorded on 200, 400 and 500 MHz spectrometers (Model No. D 205/ 52-2382, Avance 500) with TMS as the internal standard. Chemical shifts are expressed in parts per million (δ ppm). Silica gel coated aluminium plates were used for TLC. The products were purified by column chromatography on silica gel (60-120/100-200 mesh) using petroleum ether–ethyl acetate as the eluent to obtain the pure products. Elemental analyses were performed on Elementar. Reagents used were mostly purchased from Sigma Aldrich. LCMS was recorded on waters (Model No. Symapt MS) and optical rotation was reported from Perkin Elmer (Model No. 241). The molecular formulas of compounds were determined by HRMS (agilent, Model No. 6540), and all tested compounds yielded data consistent with purity $\geq$ 95% measured by by HPLC (agilent 1260 affinity)

Synthesis of Acetic acid 2-acetoxymethyl-6-allyl-3,6-dihydro-2H-pyran-3-yl ester (13): $BF_3.OEt_2$ (4.4 mL, 1.2 equiv. diluted with DCM until 15 mL) was injected to a solution of tri-O-acetylated-D- glucal (A) (10 gm, 0.036 mmol) and allyltrimethylsilane (7.05 mL, 1.2 equiv) in dry DCM at -10 ^{0}C. The reaction mixture was stirred for 2h till TLC monitoring indicated the completion of the reaction. The reaction mixture was diluted with water and extracted with DCM (3 x100 ml). The organic layers were combined and washed with aqueous sodium bicarbonate solution and brine prior to drying over anhydrous Na_2SO_4. The crude oily product obtained after filtration and concentration under reduced pressure was subjected to flash column chromatography (1-8% EtOAc in petroleum ether) to afford 13 (8.31 gm, 89%). ^{1}H NMR for α-major isomer (400 MHz, $CDCl_3$): δ, 5.94 (ddd, J = 1.6, 2.4, 10.4 Hz, 1H), 5.85 (dd, J = 3.2, 10.0 Hz, 1H), 5.80 (dt, J =2.4, 10.4 Hz, 1H), 5.16-5.10 (m, 3H), 4.30-4.26 (m, 1H), 4.23 (dd, J =6.4, 11.6 Hz, 1H), 4.15 (dd, J =3.2, 11.6 Hz, 1H), 3.96 (ddd, J =3.2, 6.4, 12.8 Hz, 1H), 2.50-2.43 (m,

1H), 2.36-2.29 (m, 1H), 2.09(s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 170.7, 170.3, 133.9, 132.8, 123.7, 117.5, 71.3, 69.7, 64.9, 62.8, 37.8, 21.0, 20.7. ESI-MS; 277.01 $(M+Na)^+$; Anal. Cal. for, $C_{13}H_{18}O_5$: C, 61.40; H, 7.14; Found C, 61.36; H, 7.07.

Synthesis of Toluene-4-sulfonic acid 6-allyl-3-hydroxy-3,6-dihydro-2H-pyran-2-ylmethyl ester (15)*:* To a solution of 13 (8.3 gm, 0.0326 mmol) in dry methanol (80 mL) was added NaOMe (176 mg, 0.1equiv.) and allowed to stirred for 30 minutes at room temperature. Completion of the reaction was monitored by TLC. The reaction mixture was neutralized with amberlyte (IR 120 H^+) resin followed by filtration and concentration of the solvent under reduced pressure. The product obtained was directly used for the next step without further purification. The compound 14 (5.5 gm, 0.032 mmol) was dissolved in dry Pyridine (30 mL) at 0 ^{0}C, followed by tosyl chloride (11.2gm, 1.8 equiv.) addition. The reaction mixture was stirred for 8h at rt., when TLC monitoring indicated the completion of the reaction. The reaction mixture was diluted with aqueous $CuSO_4$ solution and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4. Filtration and concentration under reduced pressure afforded the crude product which was subjected to purification using silica gel chromatography (5-20% EtOAc in petroleum ether) to afford 15 (6.81 gm, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79-7.77 (m, 2H), 7.33-7,31 (m, 2H), 5.79-5.77 (m, 1H), 5.76 (dd, *J* = 0.8, 3.2 Hz, 1H), 5.74-5.71(m, 1H), 5.07(d, *J* =7.6 Hz, 1H), 5.04 (dd, *J* =0.8, 9.2 Hz, 1H), 4.25 (ddd, *J* =0.8, 5.2, 11.2 Hz, 1H), 4.17 (dd, *J* =2.4, 11.2 Hz, 1H), 4.01-4.00 (m, 1H), 3.66-3.65 (m, 1H), 2.42 (s, 3H), 2.35.2.33 (m, 1H), 2.24-2.15 (m, 1H). ^{13}C NMR(100 MHz, $CDCl_3$): 145.0, 134.0, 133.6, 132.7, 129.9, 129.8, 118.4, 72.0, 69.1, 62.7, 37.7, 21.6. ESI-MS; 347.382 $(M+Na)^+$; Anal. Cal. for, $C_{16}H_{20}O_5S$; C, 59.24; H, 6.21; Found C, 59.17; H, 6.15.

Synthesis of (6-Allyl-3-hydroxy-3,6-dihydro-2H-pyran-2-yl)-acetonitrile (16). A mixture of NaCN (1.36gm, 1.5 equiv.) and 15 (6 gm, 0.0185 mmol) in dry DMSO was refluxed at 70 ^{0}C for 4h, until complete consumption of starting material as indicated by TLC. The reaction mixture was diluted with ferrous sulphate solution (to remove the excess of NaCN) and extracted with diethyl ether (3 x 40 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4, concentrated in vacuum and purified by flash chromatography (10-25% EtOAc in petroleum ether) to afford 16 (2.81gm, 85%) as a colorless powdered. ^{1}H NMR(500 MHz, $CDCl_3$): δ 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.27 (d, *J* = 6.04 Hz, 1H), 3.97-3.93 (m, 1H), 3.75 (ddd, *J* = 4.19, 7.17, 14.2 Hz), 2.78 (dd, *J* = 4.16, 16.8 Hz), 2.66 (dd, *J* =7.25, 16.8 Hz), 2.46 (m, 1H), 2.32 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$): 133.4, 130.9, 127.8, 117.9, 72.4, 69.8, 65.8, 37.8, 21.0. ESI-MS; 202.206 $(M+Na)^+$; Anal. Cal. for, $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82; Found C, 66.97; H, 7.27; N, 7.75.

General procedure for 4-hydroxyl protection of 16 (a). A solution of 16 in dry DMF at 0 ^{0}C was stirred for 30 minutes followed by addition of NaH (60% dispersion in mineral oil, 1.5 equiv.) and halide (RX) (RX = C_2H_5I, *p*-methoxybenzyl chloride, MeI, 1.2 equiv.) and stirred for 4h at rt. The reaction was completed as indicated by TLC and was diluted with ethyl acetate (50 mL) and extracted using water. The organic portion was washed with brine, dried with anhydrous Na_2SO_4, concentrated in vacuum and purified by flash chromatography.

Preparation of compound 17. Prepared by using 16 (1g, 5.58mmol), EtI (871µL, 1.2equiv.) following general procedure a to afford 17 (1.04g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ, 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.27 (d, *J* = 6.0 Hz, 1H), 3.97-3.93 (m, 1H), 3.75 (ddd, *J* = 4.1, 7.1, 14.2 Hz, 1H), 3.73-3.65 (m, 1H), 3.50-3.44 (m, 1H), 2.78 (dd, *J* = 4.1, 16.8 Hz, 1H), 2.66 (dd, *J* = 7.2, 16.8 Hz, 1H), 2.46 (m, 1H), 2.32 (m, 1H).^{13}C NMR(100MHz, $CDCl_3$): 133.4,

130.9, 127.8, 117.9, 117.4, 78.3, 72.4, 69.8, 65.8, 37.8, 21.0, 15.5. ESI-MS; 230 $(M+Na)^+$; Anal. Cal. for, $C_{12}H_{17}NO_2$: C, 59.54; H, 8.27; N, 6.76; Found C, 59.48; H, 8.14; N, 6.65.

Preparation of compound 33. Prepared by using 16(800mg, 4.46mmol), MeI(761μL, 1.2equiv.), following general procedure a to afford 33(784mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.27 (br. d, *J* = 11.2 Hz, 1H), 4.03 (ddd, *J* = 2.4, 9.2, 22.4 Hz, 1H), 3.56 (dd, *J* = 1.2, 8.8 Hz, 1H), 3.43 (s, 3H, -OCH_3), 2.78 (dd, *J* = 4.1, 16.8 Hz, 1H), 2.66 (dd, *J* = 7.2, 16.8 Hz, 1H), 2.46 (m, 1H), 2.32 (m, 1H).^{13}C NMR: 133.4, 130.9, 127.8, 117.9, 117.4, 78.3, 72.4, 69.8, 65.8, 37.8, 21.0, 15.5. ESI-MS; 216.232 $(M+Na)^+$; Anal. Cal. for, $C_{11}H_{15}NO_2$: C, 59.54; H, 8.27; N, 6.76; Found C, 59.48; H, 8.14; N, 6.65.

Preparation of compound 34. Prepared by using 16 (1g, 5.58 mmol), PMBCl(1.04 mL, 1.2 equiv.), following general procedure a to afford 34(1.46g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ, 7.24 -7.15 (m, 2H), 6.76 -6.72 (m, 2H), 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.62 (d, *J* =11.2 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.27 (dt, *J* = 2.1, 9.1 Hz, 1H), 4.07 (ddd, *J* = 2.3, 9.0,13.8 Hz, 1H), 3.80 (s, 3H, -OCH_3), 3.76 (dd, *J* = 1.3, 8.9 Hz, 1H), 2.78 (dd, *J* = 4.1, 16.8 Hz), 2.66 (dd, *J* =7.2, 16.8Hz), 2.46-2.43 (m, 1H), 2.33-2.30 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$): 159.8, 129.8, 128.6, 128.4, 117.8, 116.8, 133.4, 130.9, 127.8, 117.9, 117.4, 78.3, 72.4, 69.8, 65.8, 56.7, 37.8, 21.0. ESI-MS; 230.117 $(M+Na)^+$; Anal. Cal. for, $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.08; Found C, 72.16; H, 6.99; N, 4.00.

General procedure for cynide hydrolysis (b). Aqueous 8N NaOH solution was added to a solution of glycal cyanide in EtOH and refluxed overnight. The reaction was neutralized using 3N HCl solution after cooling to rt. The mixture was diluted with water and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuum and purified by flash chromatography.

Preparation of compound 18. Prepared by using 17 (1 g, 4.83 mmol), following general procedure b to afford 18 (818mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.27 (d, *J* = 6.0 Hz, 1H), 3.97-3.93 (m, 1H), 3.75 (ddd, *J* =4.1, 7.1, 14.2Hz, 1H), 3.73-3.65 (m, 1H), 3.50-3.44 (m, 1H), 2.78 (dd, *J* =4.1, 16.8 Hz, 1H), 2.66 (dd, *J* =7.2, 16.8 Hz, 1H), 2.46 (m, 1H), 2.32 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$):177.9, 133.4, 130.9, 127.8, 117.9, 117.4, 78.3, 72.4, 69.8, 65.8, 37.8, 33.8, 15.5. ESI-MS; 249.259 $(M+Na)^+$; Anal. Cal. for, $C_{12}H_{18}O_4$: C, 63.70; H, 8.02; Found C, 63.62; H, 7.94.

Preparation of compound 35. Prepared by using 33 (800 mg, 4.14 mmol), following general procedure b to afford 35 (614mg, 70%). ^{1}H NMR(400 MHz, $CDCl_3$): δ 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.27 (br.d, *J* = 11.2 Hz, 1H), 4.03 (ddd, *J* = 2.4, 9.2, 22.4 Hz, 1H), 3.56 (dd, *J* = 1.2, 8.8 Hz, 1H), 3.43 (s, 3H, -OCH_3), 2.78 (dd, *J* =4.1, 16.8 Hz, 1H), 2.66 (dd, *J* =7.2, 16.8 Hz, 1H), 2.46 (m, 1H), 2.32 (m, 1H).^{13}C NMR (75MHz, $CDCl_3$): 177.9, 133.4, 130.9, 127.8, 117.9, 117.4, 78.3, 72.4, 69.8, 65.8, 37.8, 36.5. ESI-MS; 235.232 $(M+Na)^+$; Anal. Cal. for, $C_{11}H_{15}NO_2$: C, 62.25; H, 7.60; Found C, 62.17; H, 7.54.

Preparation of compound 18. Prepared by using 34 (1.4g, 4.68mmol), following general procedure b to afford 36 (1.21g 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ, 7.24 -7.15 (m, 2H), 6.76 -6.72 (m, 2H), 6.00-5.83 (m, 3H), 5.17-5.12 (m, 2H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.27 (dt, *J* = 2.1, 9.1 Hz, 1H), 4.07 (ddd, *J* = 2.3, 9.0,13.8 Hz, 1H), 3.80 (s, 3H, -OCH_3), 3.76 (dd, *J* = 1.3, 8.9 Hz, 1H), 2.78 (dd, *J* = 4.1, 16.8 Hz, 1H), 2.66 (dd, *J* =7.2, 16.8 Hz, 1H), 2.46 (m, 1H), 2.32 (m, 1H). ^{13}C NMR (100MHz, $CDCl_3$): δ, 159.8, 129.8, 128.6, 128.4, 117.8, 116.8, 133.4, 130.9, 127.8, 117.9, 117.4, 78.3, 72.4, 69.8, 65.8, 56.7, 37.8, 21.0, ESI-MS; 230.116 $(M+Na)^+$; Anal. Cal. for, $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.08; Found C, 72.16; H, 6.99; N, 4.00

General procedure for the synthesis of alkenols (27-32) (c). $FeCl_3$ (5 mol %.) was added to a mixture of aldehydes (21-26, 1 equiv.) and allyltrimethylsilane (1.1 equiv.) in dry DCM at 0 °C. After completion of reaction as monitored by TLC, the resulting mixture was quenched with aqueous sodium bicarbonate and extracted with ethyl acetate (3×10 mL). The combined organic layers were dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 4:1) to afford pure products (27-32).

General procedure for EDC.HCl mediated esterification (d): A solution of acid compounds (18, 35, 36), DMAP (0.1 equiv.), and alkenols (19, 27-32) in dry DCM (0.3 M) was cooled to 0 ^{0}C and then treated with EDC hydrogen chloride (1.2 equiv.). The reaction mixture was stirred at 0 ^{0}C for 2h and then at 25 ^{0}C for further 12h. The solution was concentrated to dryness in vacuo and the residue was taken up in EtOAc and water. The organic layer was separated, washed with saturated NH_4Cl solution and dried with anhydrous Na_2SO_4. The solvent was concentrated in vacuo and purified by using flash chromatography. The products were obtained as a colorless oily liquids (20, 37-42) with 75-86% yields.

General Procedure for synthesis of the compounds 4-10 by ring closing metathesis (e). To a 3 mM solution of dienes (20, 37-42) in CH_2Cl_2 was added Grubb's-II generation catalyst (5 mmol %) under standardized reaction conditions. The reaction mixture was stirred for 8h at 0 -20 ^{0}C under N_2 atmosphere. After the reaction was complete (approximately after 2 h at rt, monitored by TLC), the reaction mixture was treated with DMSO (50 equiv, relative to catalyst) stirred overnight under air to decompose the catalyst,[29] and filtered. After removal of the solvent in vacuum followed by silica gel chromatography (2-10% EtOAc in petroleum ether) to affords **4-10** as a syrupy compounds.

Synthesis of macrolide 4: Prepared by the general procedure e using 20 (60 mg, 0.214 mmol) to afford 4 (37.03 mg, 68%) as a white syrupy. R_f 0.6 (20% EtOAc in petroleum ether): $[\alpha]^{25}{}_D$ + 6.8 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86-5.83 (d, *J* =10.4 Hz, 1H), 5.79 (ddd, *J* = 2.0, 2.4, 6.4 Hz, 1H), 5.54-5.48 (m, 1H), 5.42-5.34 (m, 1H), 5.09 (dd, *J* =2.0, 6.4, 12.8 Hz), 4.20 (br.d, *J* = 11.6 Hz,1H), 3.79 (t, *J* = 9.6 Hz, 1H), 3.73-3.65 (m, 1H), 3.54 (dd, *J* = 1.6, 8.4 Hz,1H), 3.50-3.44 (m, 1H), 2.77 (dd, *J* = 1.6, 13.6 Hz, 1H), 2.28-2.23 (m, 2H), 2.14-2.10 (m, 2H), 2.77 (dd, *J* = 1.6, 13.6 Hz, 1H), 2.28-2.23 (m, 2H), 2.14-2.10 (m, 2H), 1.25 (d, *J* = 6.4 Hz, 3H), 1.21 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 171.5, 134.1, 131.2, 126.0, 125.1, 73.9, 71.4, 71.1, 67.9, 64.4, 39.1, 35.5, 35.1, 31.0, 22.5, 15.5. HRMS $(M+H)^+$ Calc. 281.17474; Found 281.17434 ; Anal. Cal. for, $C_{16}H_{24}O_4$: C, 68.54; H, 8.63; Found C, 68.38; H, 8.63.

Synthesis of macrolide 5: Prepared by the general procedure e using 38 (30 mg, 0.077 mmol) to afford 5 (15.54 mg, 42%) as a white syrupy. R_f 0.54 (20% EtOAc in petroleum ether). $[\alpha]^{25}{}_D$ -4.2 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ, 7.34-7.29 (m, 4H), 5.95 (td, *J* = 1.6, 10.4 Hz, 1H), 5.80 (ddd, *J* = 2.0, 2.8, 8.0 Hz, 1H), 5.77 (ddd, *J* = 2.0, 2.8, 8.0 Hz, 1H), 5.64-5.60 (m, 1H), 5.57 (dd, *J* = 3.2, 11.2 Hz, 1H), 4.26 (dt, *J* = 2.0, 11.2 Hz, 1H), 4.04 (2.4, 8.8, 13.6 Hz, 1H.), 3.76-3.68 (m, 1H), 3.64 (dd, *J* = 1.2, 8.8 Hz, 1H), 3.53-3.45 (m, 1H), 2.93 (dd, *J* = 2.4, 14.8 Hz, 1H), 2.87-2.76 (m, 2H), 2.30 (dd, *J* = 12.0, 15.2 Hz ,1H), 2.25-2.20 (m,1H), 1.93 (br.d, *J* = 13.6 Hz, 1H), 1.24 (t, *J* = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, 169.6, 139.2, 136.5, 133.8, 131.4, 130.3, 128.9, 128.7, 128.5, 127.7, 126.3, 74.2, 73.9, 73.5, 69.1, 64.8, 37.8, 34.9, 29.8, 15.5. HRMS $(M+H)^+$ Calc. 363.13576; Found 363.12897; Anal. Cal. for, $C_{20}H_{23}ClO_4$: C, 66.20; H, 6.39; Found C, 66.13; H, 6.33.

Synthesis of macrolide 6: Prepared by the general procedure e by using 37 (35 mg, 0.083 mmol) to afford 6 (10.2 mg, 66%) as a white syrupy. R_f 0.48 (20% EtOAc in petroleum ether).

$[\alpha]^{25}_{D}$ -2.9 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48-7.45 (m, 2H), 7.26-7.24 (m, 2H), 5.98 (br.d, *J* = 10.4 Hz, 1H), 5.86 (br.d, *J* = 10.4 Hz, 1H), 5.83 (ddd, *J* =2.0, 2.8, 10.4 Hz, 1H), 5.63 (m,1H), 4.27 (br.d, *J* = 11.2 Hz, 1H), 4.03 (ddd, *J* = 2.4, 9.2, 22.4 Hz, 1H), 3.56 (dd, *J* = 1.2, 8.8 Hz, 1H), 3.43 (s, 3H, -OCH_3), 2.92 (dd, *J* = 2.4, 14.8 Hz, 1H), 2.81-2.64 (m, 2H), 2.31 (dd, *J* =11.6, 14.8 Hz, 1H), 2.17-2.11 (m, 1H), 1.94-1.92 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, 169.4, 139.7, 131.5, 130.5, 129.0, 128.7, 128.4, 128.1, 127.8, 126.2, 121.7, 77.0, 73.5, 72.7, 69.0, 56.5, 38.3, 36.8, 27.2. HRMS-ESI $(M+H)^+$ Calc. 393.06959; Found 363.06164 ; Anal. Cal. for, $C_{19}H_{21}BrO_4$: C, 58.03; H, 5.38; Found C, 57.96; H, 5.32.

Synthesis of macrolide 7: Prepared by the general procedure e using 39 (72 mg, 0.184 mmol) to afford 7 (27.3 mg, 41%) as a white syrupy. R_f 0.48 (20% EtOAc in petroleum ether). $[\alpha]^{25}_{D}$ -3.7 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ, 7.35-7.32 (m, 2H), 7.26-7.23 (m, 2H), 7.04-7.01 (m, 2H), 6.88-6.86 (m, 2H), 5.99 (br.d , *J* = 10.8 Hz, 1H), 5.81-5.80 (m, 1H), 5.79-5.78 (m, 1H), 5.66-5.61 (m, 1H), 5.55 (dd, *J* = 3.2, 11.2 Hz, 1H), 4.62 (d, *J* =11.2 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.27 (dt, *J* = 2.1, 9.1 Hz, 1H), 4.07 (ddd, *J* = 2.3, 9.0,13.8 Hz, 1H), 3.80 (s, 3H, -OCH_3), 3.76 (dd, *J* = 1.3, 8.9 Hz, 1H), 2.90 (dd, *J* = 2.4, 15.0 Hz , 1H), 2.83-2.79 (m, 2H), 2.24-2.17 (m, 2H), 1.59 (d, *J* =8.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ, 169.6, 163.6, 161.1, 159.4, 136.5, 131.3, 130.4, 129.7, 129.6, 129.0, 128.2, 128.1, 126.4, 115.4, 115.2, 113.9, 73.9, 73.6, 73.4, 70.9, 68.3, 53.4, 37.8, 36.2, 29.8. ESI-MS; 461.10 $(M+Na)^+$; Anal. Cal. for, $C_{26}H_{27}FO_5$: C, 71.22; H, 6.21; Found C, 71.16; H, 6.18.

Synthesis of macrolide 8. Prepared by the general procedure e using 40 (30 mg, 0.077 mmol) to afford 8 (10.4 mg, 38%) as a yellow syrupy. R_f 0.5 (20% EtOAc in petroleum ether). $[\alpha]^{25}_{D}$ -3.9 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ, 7.35-7.31 (m, 2H), 6.89-6.86 (m, 2H), 5.93 (td, *J* = 1.6, 10.4 Hz, 1H), 5.82 (ddd, *J* = 2.0, 2.8, 8.4 Hz, 1H), 5.78 (ddd, *J* = 2.0, 2.8, 8.4 Hz,

1H), 5.65-5.63 (br.m, 1H), 5.59 (dd, *J* = 3.2, 11.2 Hz, 1H), 4.28 (dt, *J* = 2.0, 11.2 Hz, 1H), 4.06 (2.0, 8.8, 13.6 Hz, 1H), 3.80 (s, 3H, -OCH_3), 3.73-3.69 (m,1H), 3.65 (dd, *J* = 1.2, 8.8 Hz, 1H), 3.50-3.47 (m,1H), 2.91 (dd, *J* = 2.8, 14.8 Hz, 1H), 2.88-2.84 (m, 1H), 2.28 (dd, *J*=11.6, 14.8 Hz, 1H), 2.25-2.21 (m, 2H), 1.93 (br.d, *J* = 13.6 Hz, 1H), 1.24 (t, *J* = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 15.5, 29.8, 34.8, 38.0, 53.4, 64.8, 69.8, 73.9, 74.0, 113.9, 126.7, 127.8, 128.0, 128.5, 130.2, 130.3, 131.8, 132.1, 132.9, 159.4, 169.7. HRMS-ESI $(M+H)^+$ Calc. 359.18530; Found 359.18396 ; Anal. Cal. for, $C_{21}H_{26}O_5$: C, 70.37; H, 7.31; Found C, 70.29; H, 7.25.

Synthesis of macrolide 9. Prepared by general procedure e using 41 (60 mg, 0.120 mmol) to afford 9 (24.8 mg, 44%) as a yellow syrupy. R_f 0.57 (20% EtOAc in petroleum ether). $[\alpha]^{25}{}_D$ -5.2 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ, 8.14 (d, *J* = 8.4 Hz, 1H), 7.87, (d, *J*= 10.0 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.56-7.54 (m, 2H), 7.53-7.46 (m, 2H), 6.92-6.86 (m, 3H), 6.62 (d, *J* =10.0 Hz, 1H), 6.01 (br.d, *J* = 11.2 Hz, 1H), 5.83 (dt, *J*= 2.0, 11.2 Hz, 1H), 5.78-5.76 (m, 1H), 5.61 (ddd, *J* = 3.2, 10.0, 22.8 Hz, 1H), 5.30 (br.d, *J* = 5.6 Hz, 1H), 4.64 (d, *J* = 11.2 Hz, 1H), 4,50-4.46 (m, 1H), 4.30 (br.d, *J* =10.8 Hz, 1H), 4.23 (ddd, *J* = 2.0, 11.2, 22.8 Hz, 1H), 3.81(s, 3H, -OCH_3), 3.80-3.79 (m, 1H), 2.98 (dd, *J* = 2.4, 13.2 Hz, 1H), 2.96-2.92 (m, 2H), 2.28-2.26 (m,1H), 2,25-2.17 (m, 1H), 1.57- 1.55 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.6, 159.4, 136.5, 133.7, 131.1, 130.9, 130.5, 130.0, 129.7, 129.6, 128.9, 128.4, 128.2, 126.8, 126.4, 125.7, 125.3, 123.5, 123.1, 122.9, 114.1, 113.9, 73.9, 73.4, 71.3, 70.9, 55.2, 38.0, 35.2, 29.9. ESI-MS; 493.10 $(M+Na)^+$; Anal. Cal. for, $C_{30}H_{30}O_5$: C, 76.57; H, 6.43; Found C, 76.51; H, 6.38.

Synthesis of macrolide 10. Prepared by the general procedure e by using 42 (30 mg, 0.072 mmol) to afford 10 (15.7 mg, 45%) as a yellow syrupy. R_f 0.6 (20% EtOAc in petroleum ether). $[\alpha]^{25}{}_D$ -2.8 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ, 7.74 (d, *J* = 7.6 Hz, 1H), 7.56 (d, *J*

= 7.6 Hz, 1H), 7.53-7.51 (m, 1H), 7.48-7.44 (m, 2H), 7.37-7.26 (m, 3H), 6.00 (d, *J* = 10.4 Hz, 1H), 5.92 (d, *J* = 10.4 Hz, 1H), 5.82 (ddd, *J* = 2.0, 2.8, 10.4 Hz, 1H), 5.67 (m, 1H), 5.58 (ddd, *J* = 3.2, 11.2, 22.4 Hz, 1H), 4.25 (dd, *J* = 2.0, 9.2 Hz, 1H), 4.08 (ddd, *J* =2.4, 9.2, 22.4Hz, 1H), 3.58 (dd, *J* =1.2, 8.8Hz, 1H), 3.44 (s, 3H, -OCH_3), 3.00-2.95 (m, 3H), 2.37-2.30 (m, 2H), 1.94-192 (m, 1H). ^{13}C NMR(100 MHz, $CDCl_3$): δ, 169.4, 140.7, 139.8, 139.3, 131.8, 131.1, 131.0, 130.4, 128.9, 128.1, 127.3, 126.4, 126.0, 125.8, 125.4, 125.2, 75.6, 73.7, 68.8, 56.5, 37.8, 34.9, 29.6. HRMS-ESI $(M+H)^+$ Calc. 459.17777; Found 459.17726; Anal. Cal. for, $C_{26}H_{25}F_3O_4$: C, 68.11; H, 5.50; Found C, 68.03; H, 5.43.

General procedure the synthesis of macrolides 11, 12 by deprotection of PMB ethers (f).To a stirring solution of macrolides 7, 9 in CH_2Cl_2/H_2O (9:1) was added DDQ (3 equiv.). The resulting mixture was stirred for 45 min. at rt. The mixture was poured into saturated aqueous $NaHCO_3$ solution and further extracted with DCM (3 x 10mL). The combined organic layers were washed with brine and dried with anhydrous Na_2CO_3. The solvent was concentrated in vacco and purified by flash column chromatography (15-30% EtOAc in petroleum ether) to affords 11, 12 with the yields of more than 90%.

Synthesis of macrolide 11. Prepared by the general procedure f by using (30 mg, 0.082 mmol) to afford 11 (19.7 mg, 91%) as a white powder. R_f 0.5 (50% EtOAc in petroleum ether). mp: 130-132; $[\alpha]^{25}_{D}$ -4.5 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ, 7.38-7.345(m, 2H), 7.06-7.02 (m, 2H), 5.99 (br.d, *J* = 10.8 Hz, 1H), 5.81-5.80 (m, 1H), 5.79-5.78 (m, 1H), 5.66-5.61 (m, 1H), 5.55 (ddd, *J* = 3.2, 11.2, 22.6Hz, 1H), 4.62 (d, *J* =11.2 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.27 (dt, *J* = 2.1, 9.1 Hz, 1H), 4.07 (ddd, *J* = 2.3, 9.0,13.8 Hz, 1H), 3.76 (dd, *J* = 1.3, 8.9 Hz, 1H), 2.83-2.79 (m, 2H), 2.24-2.17 (m, 2H), 1.59 (d, *J* =8.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.6, 161.1, 136.5, 129.7, 129.6, 129.0, 128.2, 126.4, 115.4, 113.9, 73.9, 73.6, 73.4,

70.9, 68.3, 37.8, 36.2, 29.8. HRMS-ESI $(M+H)^+$ Calc. 319.13401; Found 319.13369; Anal. Cal. for, $C_{18}H_{19}FO_4$: C, 67.91; H, 6.02; Found C, 67.83; H, 5.97.

Synthesis of macrolide 12. Prepared by the general procedure f by using 9 (25 mg, 0.053 mmol) to afford 12 (17.1 mg, 92%) as a white powder. R_f 0.6 (50% EtOAc in petroleum ether). mp: 157-159 ^oC; $[\alpha]^{25}{}_D$ -4.5 (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ, 8.14 (d, *J* = 8.4 Hz, 1H), 7.87, (d, *J* = 10.0 Hz, 1H), 7.80 (d, *J* = 8.0, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.53-7.51 (m, 1H), 7.48- 7.26 (m, 2H), 6.01 (br.d, *J* = 11.2 Hz, 1H), 5.83 (dt, *J* = 2.0, 11.2 Hz, 1H), 5.78-5.76 (m, 1H), 5.61 (ddd, *J* = 3.2, 10.0, 22.8 Hz, 1H), 5.30 (br.d , *J* = 5.6 Hz, 1H), 4.64 (d, *J* = 11.2 Hz, 1H), 4.50-4.46 (m, 1H), 4.30 (d, *J* =10.8 Hz, 1H), 4.23 (ddd, *J* = 2.0, 11.2, 22.8 Hz, 1H), 3.80-3.79 (m, 1H), 2.98 (dd, *J* = 2.4, 13.2 Hz, 1H), 2.96-2.92 (m, 2H), 2.28-2.26 (m,1H), 2.25-2.17 (m, 1H), 1.57- 1.55 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.6, 136.5, 133.7, 131.1, 130.9, 130.5, 130.0, 129.7, 129.6, 128.2, 126.8, 126.4, 125.7, 125.3, 123.1, 122.9, 73.9, 73.4, 71.4, 70.9, 38.0, 35.2, 29.9. HRMS-ESI $M+H)^+$ Calc. 351.15909; Found 351.15911 ; Anal. Cal. for, $C_{22}H_{22}O_4$: C, 75.41; H, 6.33; Found C, 76.36; H, 6.27.

ASSOCIATED CONTENT

Supporting Information. ^1H NMR, ^{13}C NMR, HRMS spectra for compounds **4-12** and copounds 13-16. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATIONS

***Corresponding Author**. Tel.:+91-091-2569000; fax: +91-091-2569111; e-mail: dmukherjee@iiim.ac.in, fmalik@iiim.ac.in. ! IIIM communication No. 1512

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ABBEREVIATIONS USED

DHP, dihydro pyran; EDC, 1-ethyl-3-(3 dimethylamino propyl) carbodimide; PI3K, phosphotidy inositol 3-phosphate.

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Graphical abstract: