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SYNTHESIS AND BILOGICAL EVALUATION OF ASPERGILLIDE ANALOGUE AS ANTICANCER AGENTS

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ABSTRACT:-*Three novel bicyclic 14-membered macrolides with 2,6-cis and trans-fused dihydro- or tetrahydropyran rings, namely aspergillides A–C, have recently been isolated by Kusumi and co-workers from a marine-derived fungus Aspergillus ostianusstrain 01F313. These compounds exhibited potent cytotoxicity against mouse lymphatic leukemia cells and also toward a number of different cancer cell lines including, HL-60 (human promyelocyticleukemia), MDA-MB-231 (human breast carcinoma), and HT1080 (human fibrosarcoma) cell lines.*

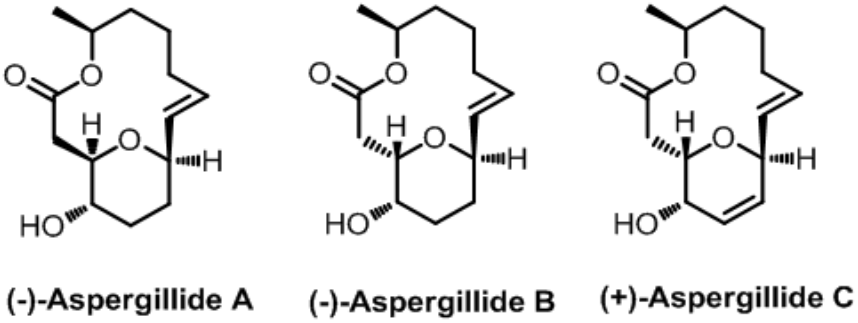


Fig 1 aspergillides

Altering the core skeleton and substituents on free alcohol of aspergillides resulted in improved biological activity[1- 8]. *Based on the biological evaluation results and as part of our ongoing projects in this area we became interested to design and synthesize some analogues by altering the core skeleton and the substituents on pyran ring alcohol which are expected to be more potent than the parent aspergillide. Objective of the current work is to prepare novel**13** membered aspergillide analogues. Herein, the synthesis of some of the analogues is described. The synthesized analogues will be evaluated for their anti tumor activity against various cancer cell lines.*

Key words:*macrolide, achmatowicz rearrangement, ferrier type alkynylation*

1. INTRODUCTION

Natural products are a prolific source of lead structures for drug discovery and development and a significant fraction of current prescription drugs are either natural products themselves or have been derived from a natural product lead [9-10], either directly or indirectly. The microtubule-stabilizing natural products epothilones A/B and zampanolide, which are proven or potential leads for drug discovery in oncology. Epothilones are bacterial macrolides with potent microtubule-stabilizing and antiproliferative activity [11]. At least 8 epothilone-type agents have entered clinical trials in humans and one of these (ixabepilone, IxempraR) has approved by the FDA in 2007 for clinical use in cancer patients. The change of the natural epoxide geometry from *cis* to *trans*, the incorporation of a conformationally constrained side chain, the removal of the C3-hydroxyl group, and the replacement of C12 with nitrogen has led to analogues **3** and **4** as the most advanced structures, both of which are potent antiproliferative agents with low nM activity against several human cancer cell lines *in vitro*. Aspergillides A-C are found to be the novel anticancer drugs of natural sources.

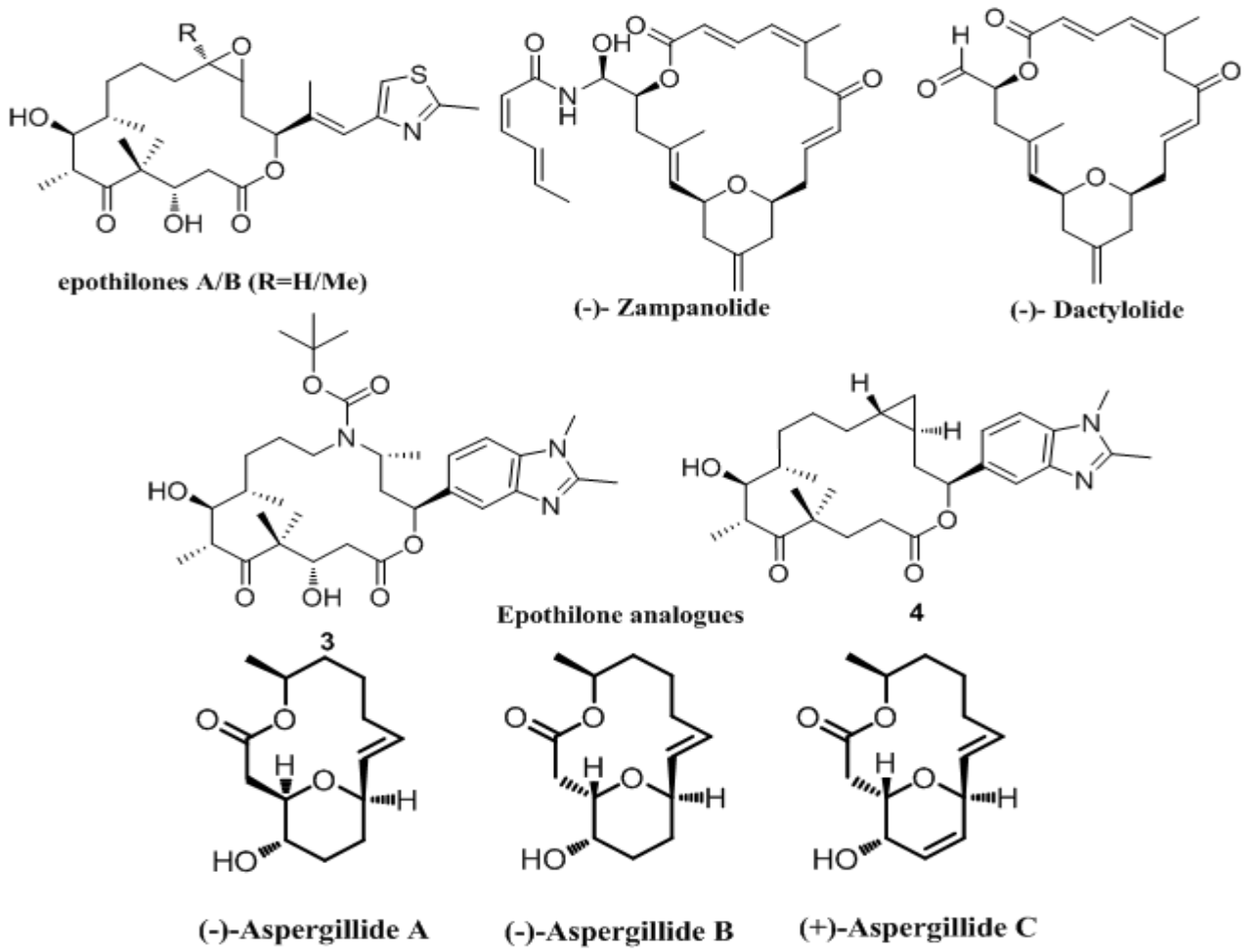


Fig 2 natural products

2. MATERIALS AND METHOD

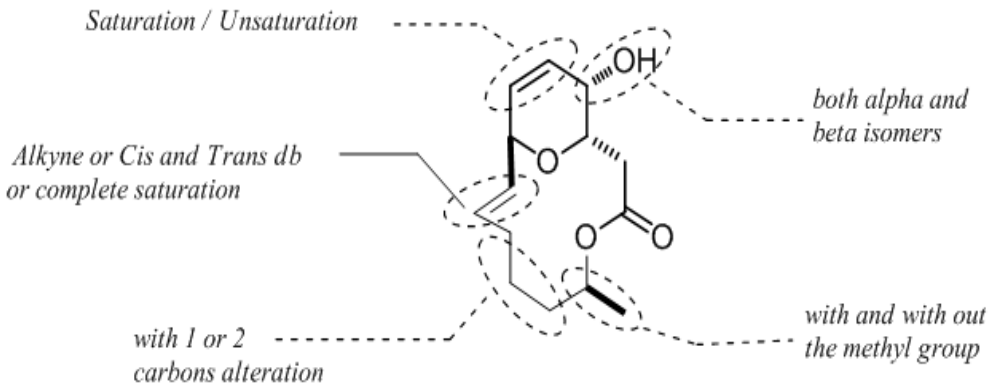
2.1 Materials:

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$or C_6D_6 as solvent on 200 MHz or 300 MHz or 500 MHz spectrometer at ambient temperature. The coupling constant *J* is given in Hz. The chemical shifts are reported in ppm on scale downfield from TMS as internal standard and signal patterns are indicated as follows: s = singlet, d = doublet, t = triplet, q = quartet, sex = sextet, m = multiplet, br = broad. FTIR spectra were recorded on KBr pellets $CHCl_3$/neat (as mentioned) and reported in wave number (cm^{-1}).. For low (MS) resolution, *m/z* ratios are reported as values in atomic mass units. Mass analysis was done in ESI mode. All reagents were reagent grade and used without further purification unless specified otherwise. Solvents for reactions were distilled prior to use: THF, toluene and diethyl ether were distilled from Na and benzophenone ketyl; MeOH from Mg and I_2; CH_2Cl_2 from CaH_2. All air- or moisture-sensitive reactions were conducted under a nitrogen or argon atmosphere in flame-dried or oven-dried glassware with magnetic stirring. Column chromatography was carried out using silica gel (60-120 mesh or 100- 200 mesh) packed in glass columns. Technical grade ethyl acetate and petroleum ether used for column chromatography were distilled prior to use

2.2 Method:

Altering the core skeleton and/or substituents on free alcohol of aspergillides resulted in improved biological activity. Based on the biological evaluation results of aspergillides we planned to synthesize analogues by altering the core skeleton and the substituents on pyran ring alcohol. Objective of the current work is to prepare **13** membered aspergillide analogues with alkyne or *cis* or *trans* double bond on pyran ring and desmethylated / methylated analogues as well as with the α and β isomers of the allylic secondary alcohol with various substituents like Me or Bn, groups. The designed analogues will be synthesized in unified strategy that means maximum of analogues will be generated from a common single key intermediate.

Variable sites in aspergillide-C macrolide to produce different analogues:

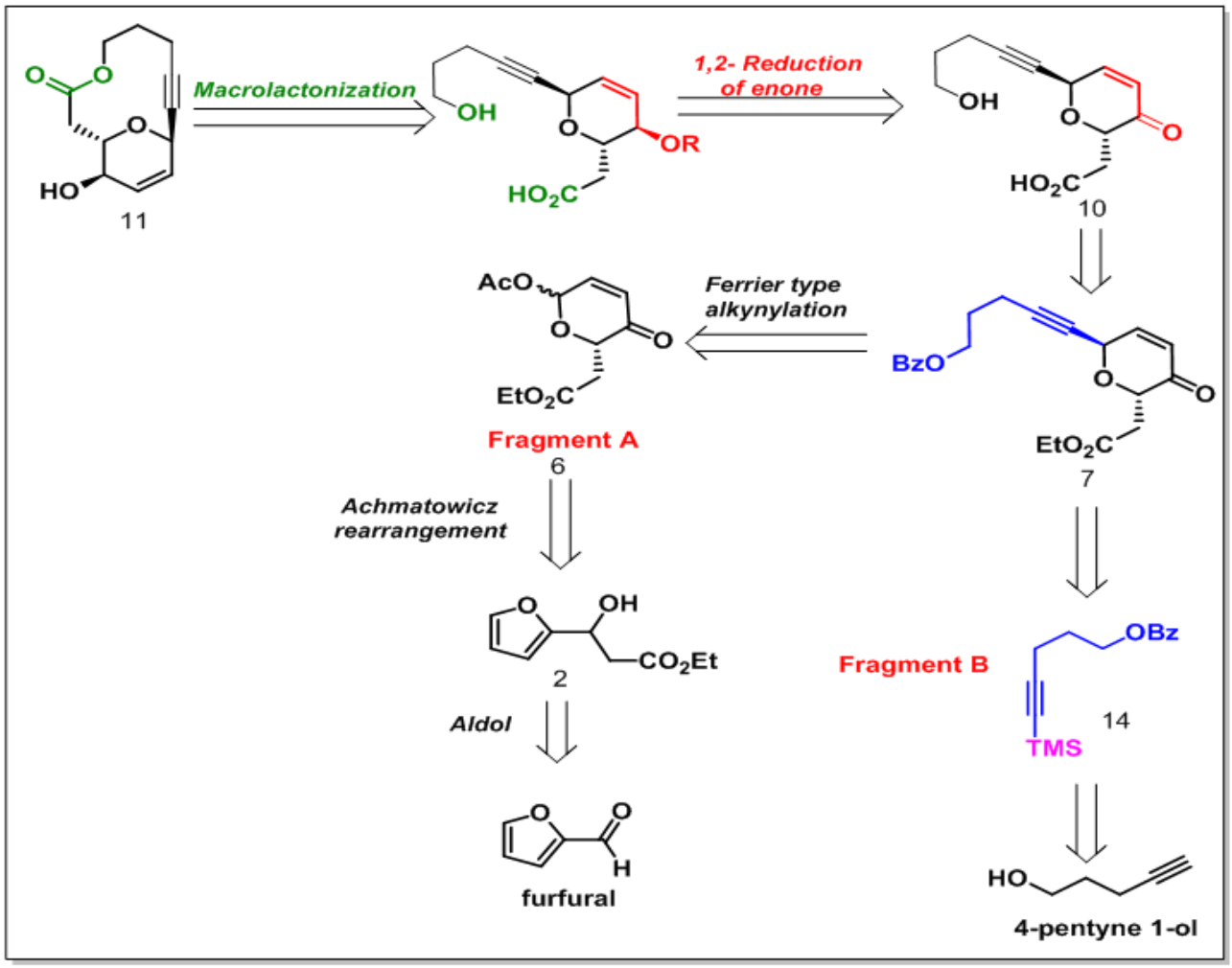


(-)-Aspergillide-C

Fig 3

Retrosynthetic analysis for the synthesis of key lactone intermediate:

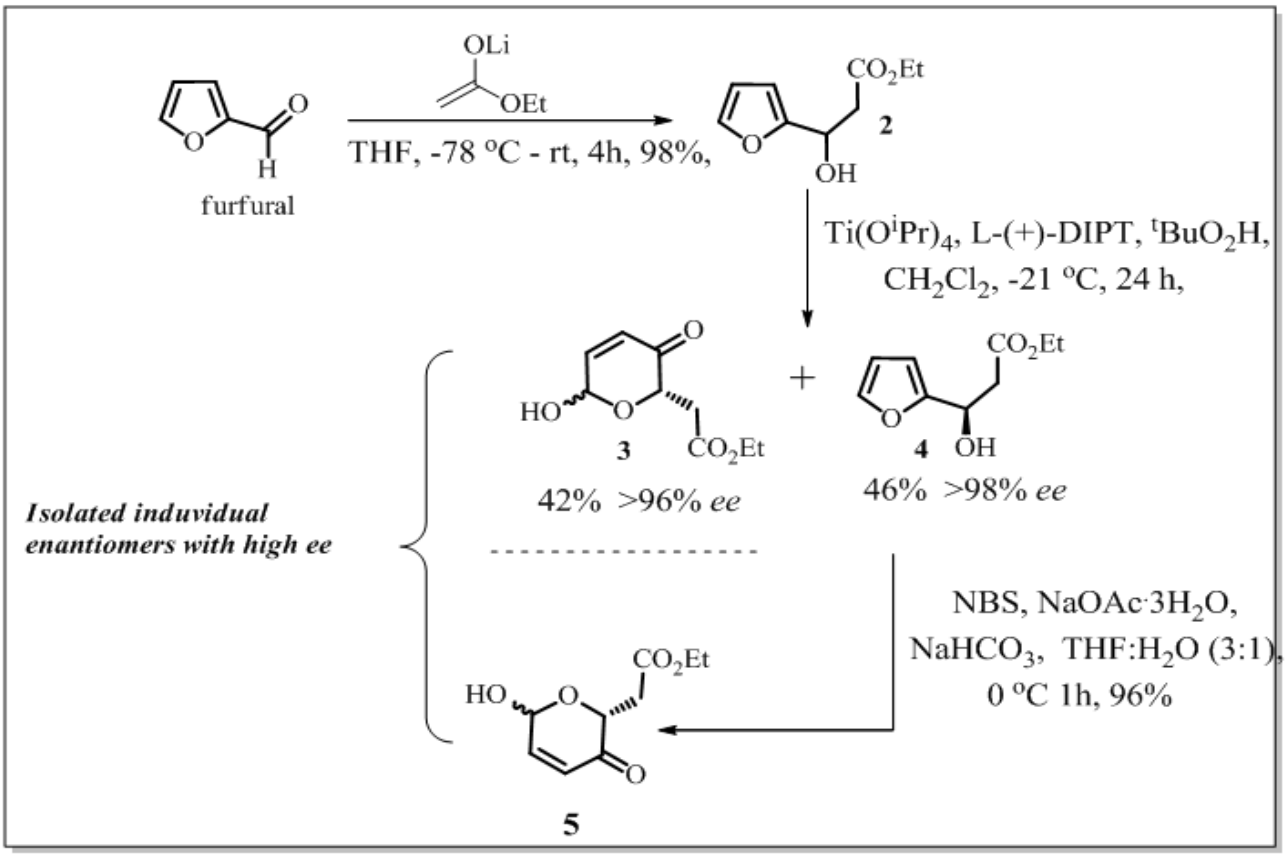
We envisaged the target macrolide 1**1** could be achieved from its corresponding seco acid **10** in two steps involving lactonization and TBS deprotection.Seco acid **10** could be synthesized from enone**7** by sequential reactions like reduction of enone to allyl alcohol and its protection with TBS then finally diester hydrolysis. Compound **7** can be accessed from acetal**6** by Ferrier type alkynylation with benzylated TMS–acetylene **14**. While acetal**6** could be obtained from the furfuryl alcohol **2**, the alkyne fragment **14** can beaccessed from commercially available 4-pentyne 1-ol.



3. SYNTHETIC SCHEME

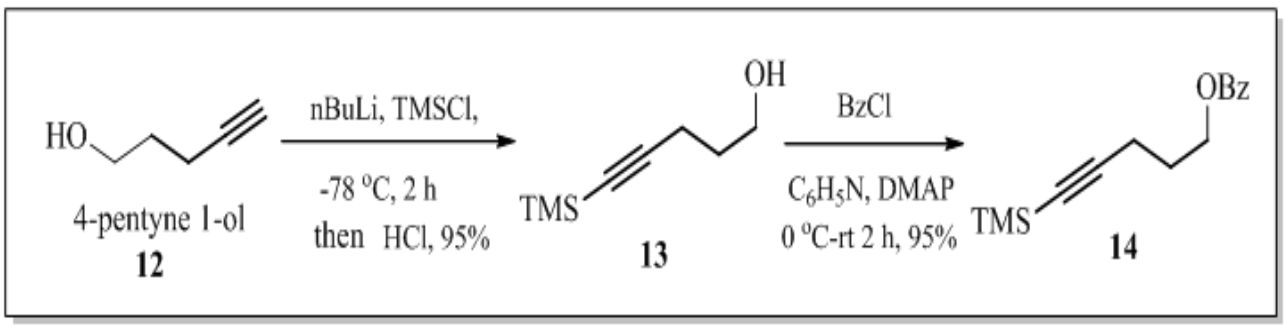
As mentioned earlier for the synthesis of macrolide analogues we needed the macrolide intermediate **11**. In order to achieve it we started our synthesis according to our retro synthetic analysis in which we first synthesized required fragments A and B then the union of two fragments gave key intermediate and some functional group manipulations finally afforded key macrolide by which the target macrolide analogue synthesized.

3.1 SCHEME:1



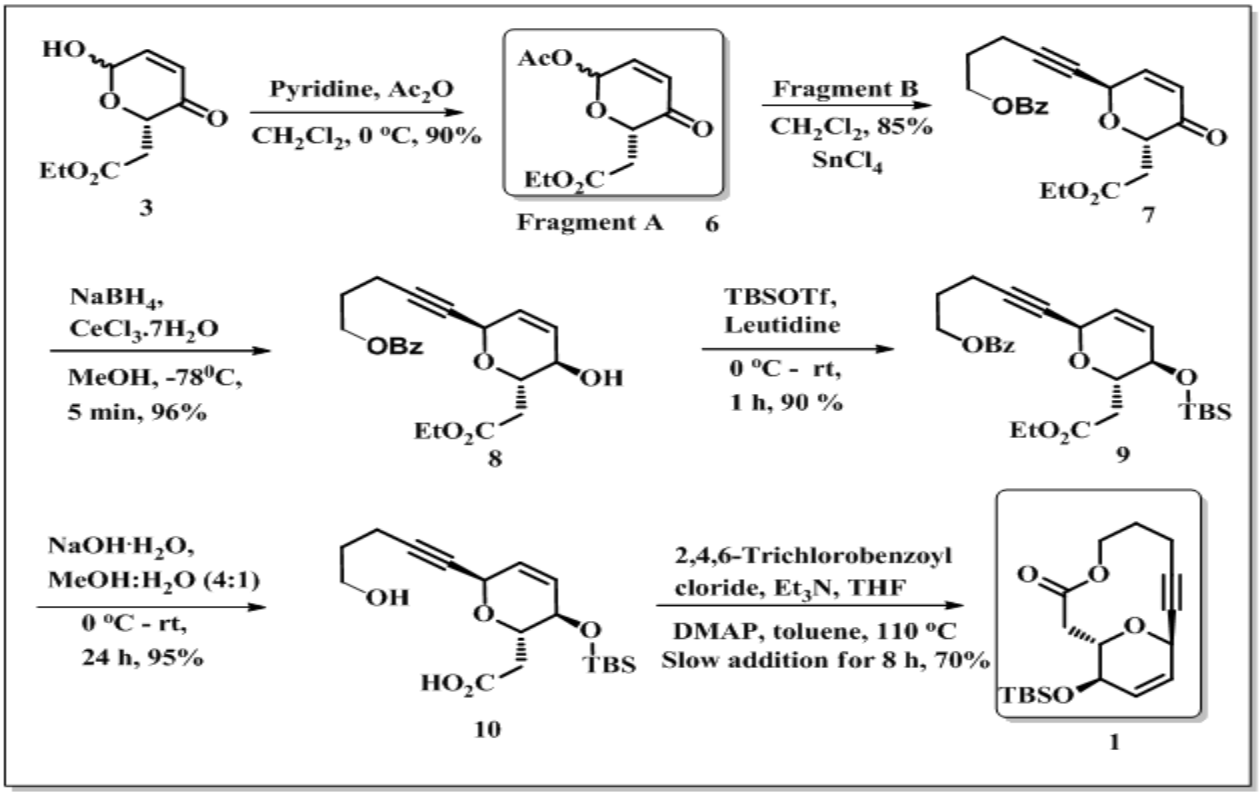
The racemic secondary furfuryl alcohol was synthesized by the aldol addition of enolate, generated from ethyl acetate, to furfuraldehyde.[16-20].

3.2 SCHEME 2



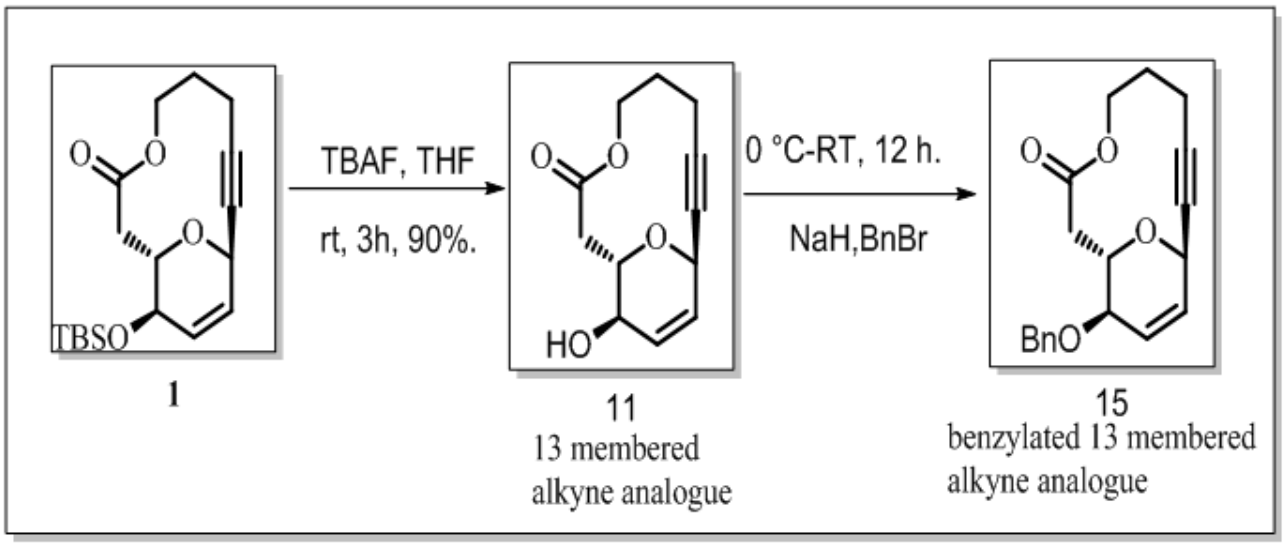
The alkyne fragment was synthesised from commercially available 4-pentyne-1-ol. The pentyne-ol treated with two equivalents of *n*BuLi to generate acetalidealkoxide di anion then it was treated with TMS Chloride to give di silylated ether (C and O silylated) then the silyl ether was selectively cleaved with 1N HCl to yield mono C-silylated alcohol **13.**TMS alcohol **13** obtained was subjected to benzoylation in Pyridine-Benzoyl Chloride system to obtain O-benzoylated TMS alkyne **14**.

3.3 SCHEME 3



The pyranonelactol**3** obtained in the above step was activated to the corresponding acetate by using acetic anhydride in presence of pyridine to give acetate **6**.The activated pyranone acetate **6** obtained was subjected to Ferrier-type alkynylation with silylated alkyne**14** in the presence of 30 mol% of strong Lewis Acid $SnCl_4$to give alkyne adduct **7** in 82% yield.Theenone**7** obtained was reduced chemoselectively to the allyl alcohol **8** under standard Luche conditions[21,22]..The free hydroxy moiety of allyl alcohol **8** obtained was subjected to O-TBS protection in the presence of TBSOTf and lutidine as a base to give the corresponding TBS ether **9.**The di-ester **9** obtained was subjected to ester hydrolysis with NaOH to produce acid alcohol **10**.Acid functionality in **10** was activated with 2 4 6-trichloro benzoylchloride, TEA to its mixed anhydride, and then this mixed anhydride was slowly added with syringe pump to refluxing toluene and DMAP to generate the macrolide **1**.

3.4 Synthesis of designed analogues from the lactone intermediate 11:



The macrolide **1** obtained in above transformation was subjected to TBS deprotection with TBAF to give 13 membered macrolide analogue **11.** The macrolide **11**obtained in the above step was subjected to benzylation by using benzyl bromide in presence of sodium hydride to give 13 membered macrolide analogue**15**.

3.5 Determination of Anticancer activity by MTT assay:

Anticancer activity has been carried out for the synthesized compounds using different cancer cell lines, HL-60 (human promyelocytic leukaemia),MCF-7(human breast carcinoma)and HT-29(human colon carcinoma) cell lines.

Cell proliferation and viability was determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. The pale yellow colored tetrazolium salt (MTT) reduces to a dark blue water-insoluble formazan by metabolically active cells and that is measured quantitatively after soluble in DMSO. The absorbance of the soluble formazan is directly proportional to the number of viable cells. Cells were seeded at a density of 1×10^4 cells in 200 µL of medium per well of 96-well plate. The 96-well microtiter plates were incubated for 24 h prior to addition of the experimental compounds. Cells were treated with vehicle alone (0.4% DMSO) or compounds (drugs were dissolved in DMSO previously) at different concentrations (1, 10 and 25µM) of test compounds for 48 hours. The assay was completed with the addition ofMTT (5 %, 10µL) and incubated for 60 min at 37 ^{0}C. The supernatant was aspirated and plates were air dried and the MTT-formazon crystals dissolved in 100 µL of DMSO. The optical density (O.D.) was measured at 560 nm using TECAN multimode reader. The growth percentage of each treated well of 96 well plate have been calculated based on test wells relative to control wells. The cell growth inhibition was calculated by generating dose response curves as a plot of the percentage of surviving cells versus drug concentration. Antiproliferative activity of the cancer cells to the test compounds was expressed in terms of IC_{50} value, which defines as a concentration of compound that produced 50% absorbance reduction relative to control.

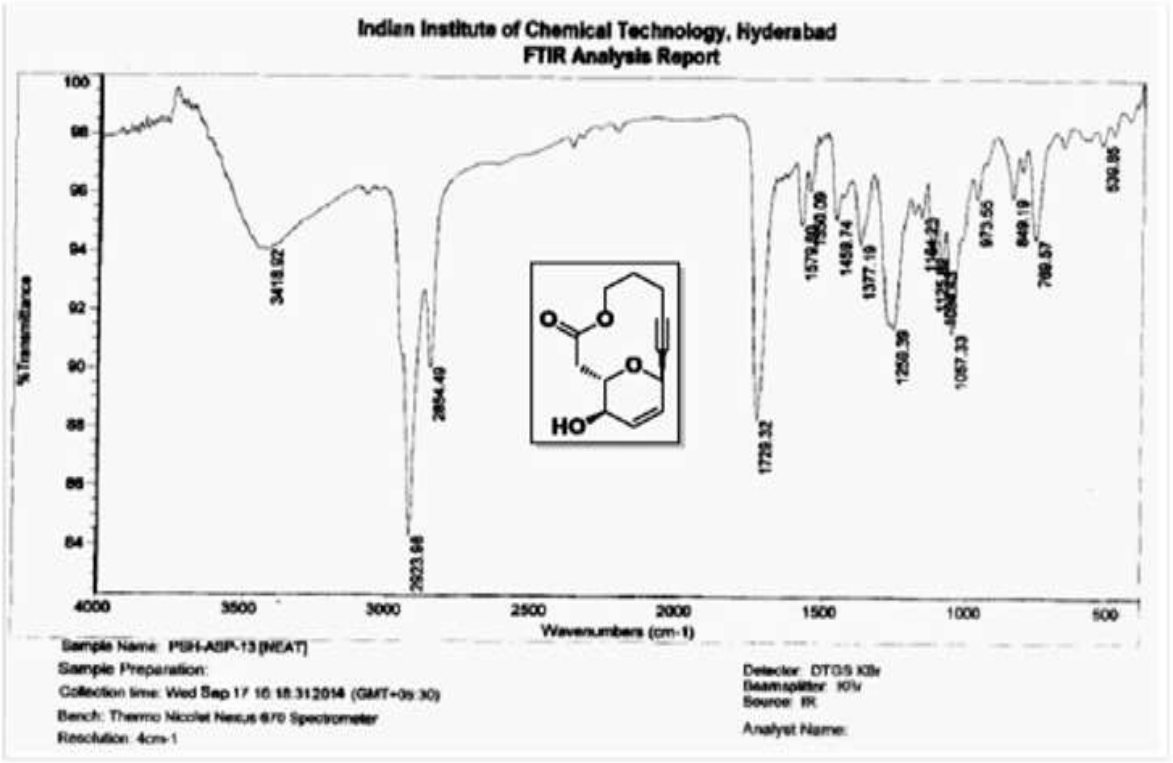
4. RESULTS AND DISCUSSION

Natural products have found use in human medicine for thousands of years. Kusumiand co-workers[16] isolated three novel bicyclic 14-membered macrolides with2,6-*cis* and *trans*-fused dihydroortetrahydropyran rings, namely aspergillides A–C, from amarine-derived fungus *Aspergillusostianus*strain 01F313, and they reported that these macrolactones shows anticancer activity. We synthesized (1*S*,10*R*,*13R)*-13-(benzyloxy)-4,14-dioxa-bicyclo [8.3.1]tetradec-11-en-8-yn-3-one(15)and all synthesized compounds analyzed by ^{1}H NMR, ^{13}C NMR, MASS and IR spectra. In order to synthesize Aspergillide analogues seco-acid was prepared and subjected to lactonisation by employing Yamaguchi protocol, then the free hydroxy moiety of macrolide was subjected to benzylation by using benzyl bromide.

General discussions on data obtained:

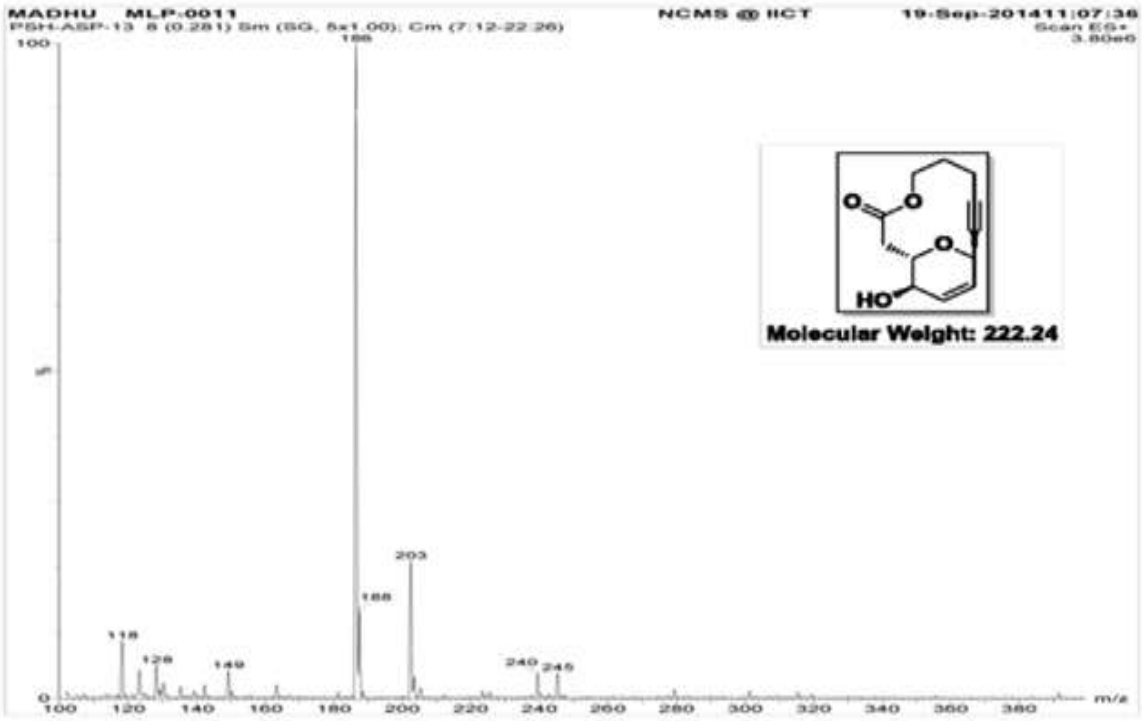
(1*S*,10*R*,13*R*)-13-Hydroxy-4,14-dioxabicyclo[8.3.1]tetradec-11-en-8-yn-3-one(11):

In IR spectrum.(Fig.4) the broad peak at 3418 (cm^{-1}) indicates the presence of hydroxy group, strong peak at 2923(cm^{-1}), 2854(cm^{-1}) are due to CH of aliphatic stretching,1729 indicates the presence of carbonyl group.ESI MASS(m/z) spectrum (Fig.5)shows 245.24 [M + 23] peak.^{1}HNMR(300 MHz, $CDCl_3$)spectrum (Fig.6)shows doublets at δ5.85 and 5.72integrating for 2 proton of unsaturated C, present in the pyran ring, singlet at δ4.80integrating assignable for 1 proton on carbon containing hydroxy moiety in pyran ring, multiplet at δ 4.53-4.37 integrating for 2 protons present on the both carbons adjacent to the oxygen of pyranring,multiplet at δ4.13-4.05integrating for 2 protons present on the carbon adjacent to the cyclic ester group,δ 2.96integrating for 1 proton, multiplet at δ2.42-2.34integrating for 3 protons,multiplet at 2.05-1.99integrating for 1 proton,multiplet at 0.98-0.84 integrating for 2 protons of alkane chain with in the ring system.^{13}C NMR (75 MHz, $CDCl_3$) (Fig.4.2.38)the spectrum had peak at δ167.1 indicates the presence of carbonyl carbon and peaks at δ 132.1, 128.0 areresponsibleforaromaticcarbons of pyran ring,δ86.6, 80.1,are responsible for alkyne carbons in the ring system, δ73.6, 66.7, 66.6, areresponsibleforpyrancarbons,δ 65.1 peak shows oxygen attached carbon in the ring system, δ 37.0, 27.2, 17.7areresponsible for alkane carbons in the ring system.The structures of compounds prepared during the present investigation have beenauthentically established by their ^{1}H NMR, ^{13}C NMR, Mass, I.R spectral studies.



IR spectroscopy data of (1*S*,10*R*,13*R*)-13-Hydroxy-4,14-dioxabicycl o [8.3.1] tetradec-11-en-8-yn-3-one(11):

Fig 4 IR spectroscopy of compound 11



Mass spectroscopy data of (1*S*,10*R*,13*R*)-13-Hydroxy-4,14-dioxa bicyclo [8.3.1] tetradec-11-en-8-yn-3-one(11):

Fig 5 Mass spectroscopy of compound of 11

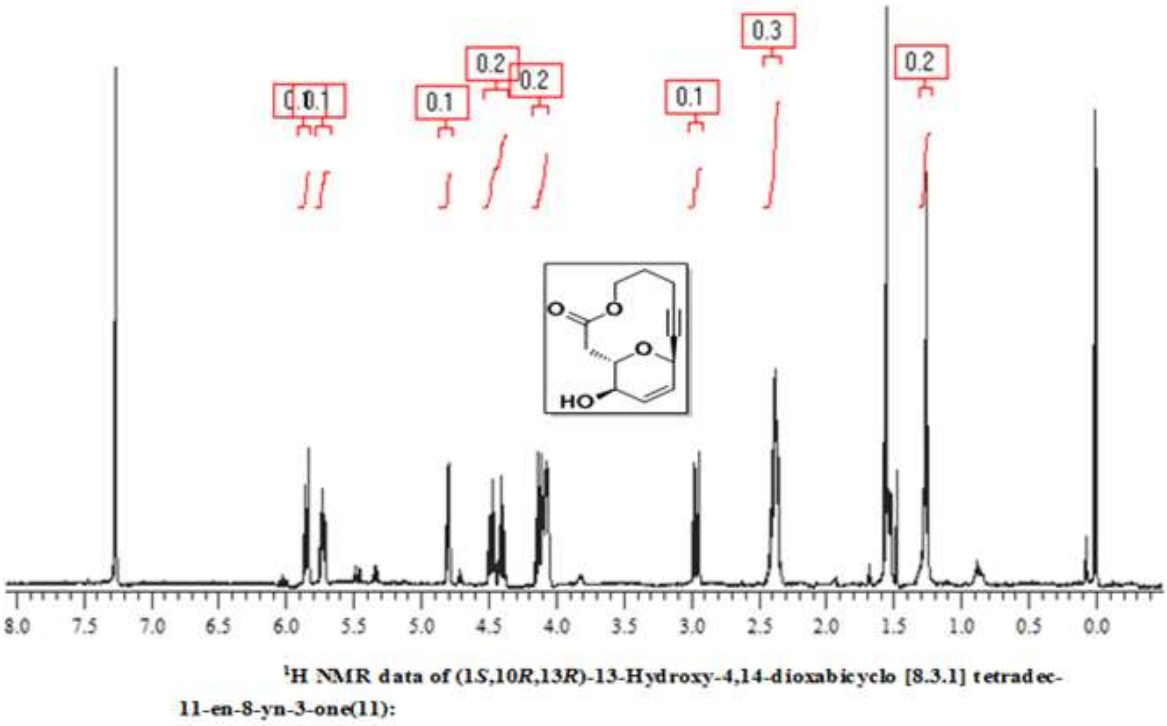


Fig 6[1]H NMR of compound 11

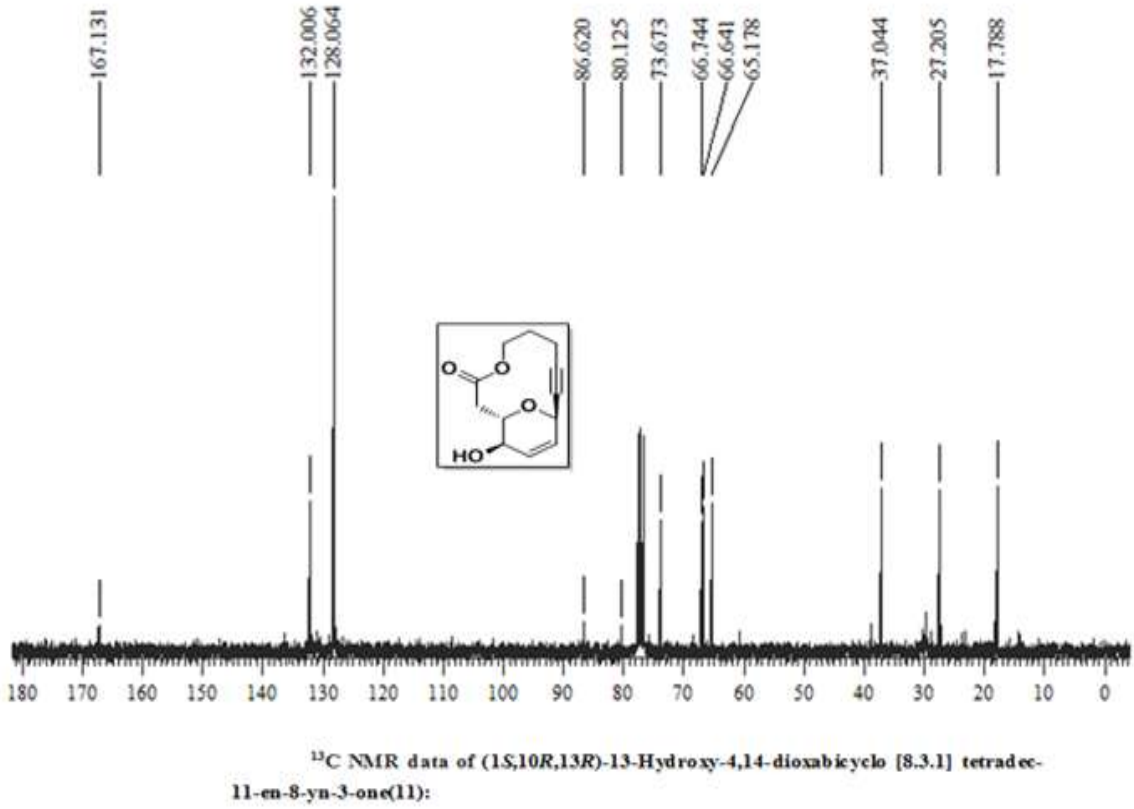
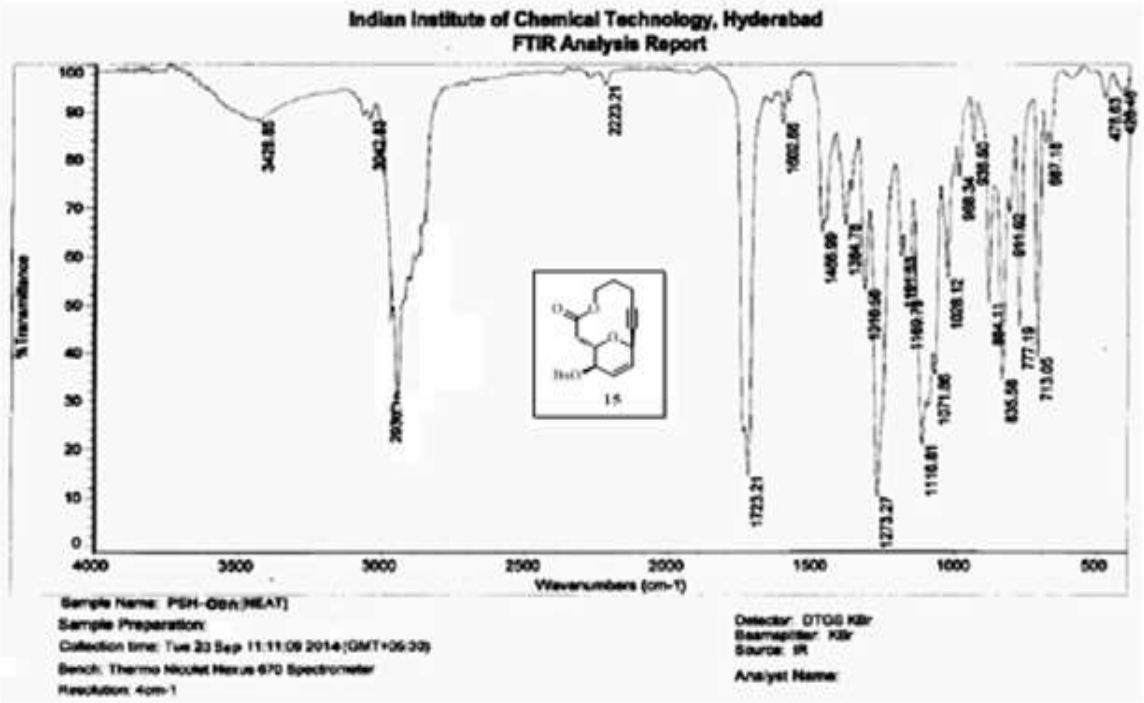
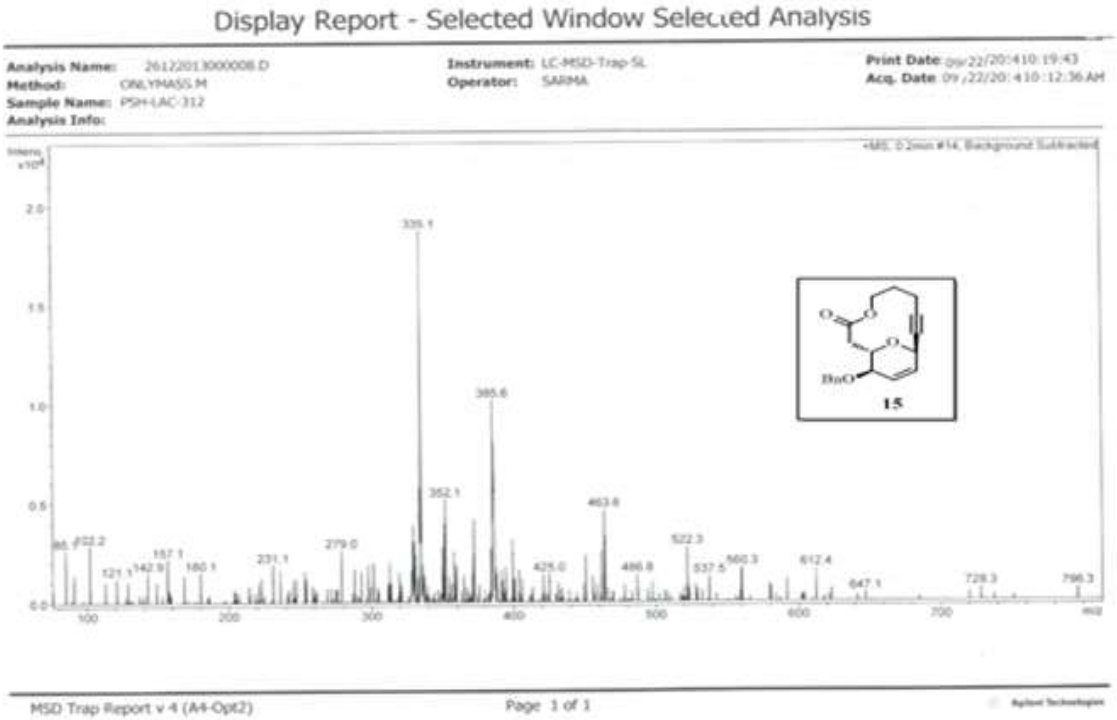


Fig 7 [13]C NMR of compound 11



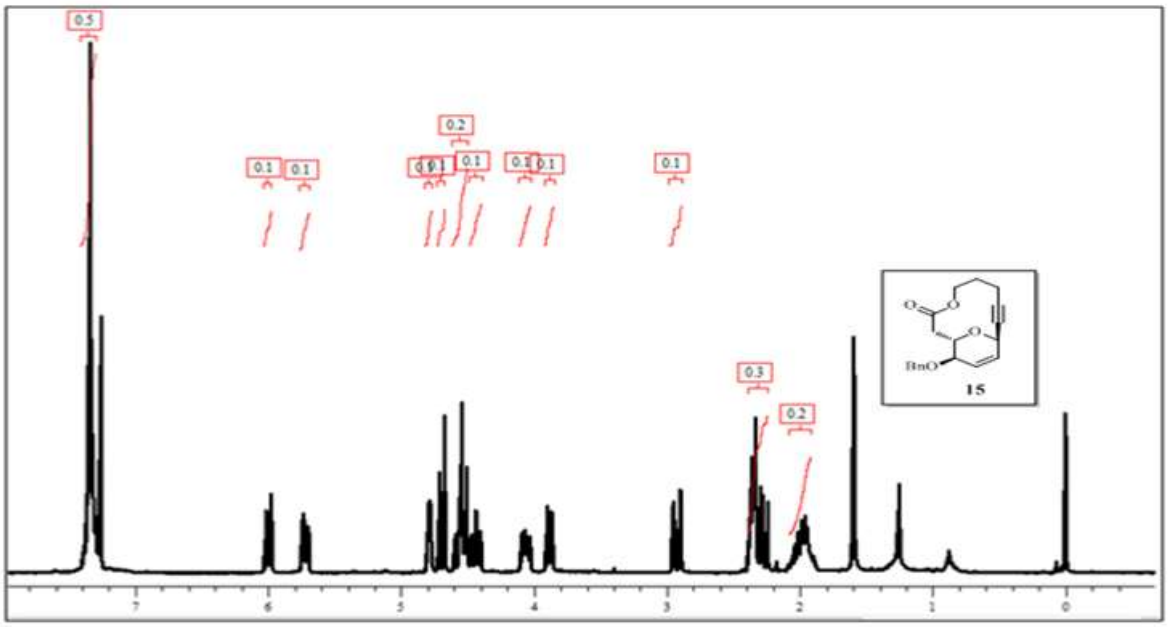
IR spectroscopy data of (1*S*,10*R*,13*R*)-13-(benzyloxy)-4,14-dioxa-bicyclo [8.3.1] tetradec-11-en-8-yn-3-one(15):

Fig 8 IR spectroscopy of compound 15



Mass spectroscopy data of (1*S*,10*R*,13*R*)-13-(benzyloxy)-4,14-dioxa-bicyclo [8.3.1]tetradec-11-en-8-yn-3-one(15):

Fig 9 Mass spectroscopy of compound of 15



^{1}H NMR data of (1*S*,10*R*,13*R*)-13-(benzyloxy)-4,14-dioxa-bicyclo [8.3.1] tetradec-11-en-8-yn-3-one(15):

Fig 10^{1}H NMR of compound 15

Anticancer activity:

Table 1 Anticancer activity

IC_{50} (µM) for the 48 h of action of investigated compounds and (std) on the HL-60, HT-29 1080,MCF-7/Dox by MTT assay			
COMPOUND	HL-60	HT-29	MCF-7
DOXARUBICIN	0.08±0.01	0.85±0.11	84.3±13.7
11	78.8±7.6	65.8±7.8	69.51±8.9
15	80.09±5.4	62.4±8.9	56.06±8.9
Each data represents mean + S.D. from 3 different experiments performed. HL60 human leukaemia cancer cell line;HT-29 colon carcinoma cell line; MCF-7/DOX breast carcinoma cell lines.			

5. CONCLUSION

Synthesis of designed analogue of Aspergillide has been achieved using key strategy reactions such as Ferrier type alkynylation, Sharpless kinetic resolution, Achmatowicz reaction, Luche reduction, Yamaguchi macrolaconization. All these compounds were purified by column chromatography and they were characterized by the analytical and spectral (TLC, ^{1}H NMR, ^{13}C NMR, IR, MASS) methods. The samples were tested against anticancer activity using MTT assay, and have been found to show a moderate activity against several cell lines(HL-60, HT-29, 1080, MCF-7 cell lines) in reference to standard drug i.e., Doxorubicin.

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