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ISOLATION OF ANTI-MALARIAL COMPOUNDS FROM ARTEMISIA ANNUA L AND SEMI-SYNTHESIS OF NOVEL DIHYDROARTEMESININ DERIVATIVES

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ABSTRACT

Artemisia annua L has been reported with various medicinal activities like Antimalarial, Anticancer, Immunosuppressive, Anti-parasitic, Anti-SARS-CoV-2, Antimicrobial, Antidiabetic, Antipyretic, Hypolipidemic, Anti-inflammatory, Anti-helmintic, Anti-asthmatic and Anti-asthmatic activities. In the quest of the compounds responsible for the above activities the plant A. annua was selected for the phytochemical investigation.The present work is to extract, isolate, purify and characterize the phenylpropanoids from the aerial parts of the plant and semi-synthesis of novel bio active Dihydroartemesinin-1,2,3-triazole analogues by click chemistry.

Keywords: *Artemisia Annua,* Artemisinin, Dihydroartemesinin, 1, 2, 3-Triazoles, Anti-Malarial, Antidiabetic, Antiulcer.

I. INTRODUCTION

Generally, medicinal plants denoted to be the one that possess some valuable constituents, genuine properties & mentioned themselves as drugs or therapeutic agents which are used to treat various diseases and disorders. Active constituents are available abundant in the most of the plant parts.

ETHNO BIOLOGICAL USES OF MEDICINAL PLANTS

The botanical medicine followed traditionally & culturally from our ancestors by using the plants which are found near by home surroundings. Some of the commonly used herbs like artichoke leaf extract, holy basil, ginger, garlic, turmeric, cinnamon, yarrow and rosemary were popularly known as cholesterol lowering medicinal plant. Some herbal spices like *cinnamon, clove* and *rosemary* found to be beneficial as blood pressure controllers and tincture of *Thyme* which is known for itsbest anti-acne properties.

Plant's primary metabolites:

Primary metabolites, are defined as molecules which are necessary in bio synthetic pathways, helps in the growth & surviving of the plant cells. Some of these metabolites can be prepared by plant itself.

Which are mentioned below **e. g.** Carbohydrate, amino - acids, fatty - acids, protein molecules, nucleic acids etc which involves in the growth and development of plant.

Secondary metabolites of Plants:

The plant's secondary metabolites are actually synthesized from basic precursors/primary metabolites and will accumulate in cellular regions. The list of secondary metabolites are discussed below

Fatty acids

Fatty acids are considered in the class of compounds in bio-chemical classification is described as a polar hydrophilic part (head) area is coupled to a lengthy lipophilic or hydrophobic hydrocarbon tail. Plants synthesize and store some for strength, unfortunately it mostly utilizes to form fatty/lipid membrane compounds orelse found as the composition in the cell membrane of protoplast. The fatty acids which are most commonly found in plants include Oleic acid (it isa saturated hydrocarbon chain) and Palmitic acid (an unsaturated molecule having double bonds in its long chain).

Terpenes

This compounds are considered to be a different class of compounds in the natural Phyto-chemical division & they are natural constituents which are hydrocarbon-based, derives their structural form from the Isoprene believed asbackbone and later on it is divided into Iso-pentane (2-methylbutane) units.

Phenols

Presence of phenolic groups in the plants gives the pleasant/aromatic odour and the essence nature to the

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plant.Surprisingly, the phenolic groups present in the aromatic plants doesn't exist in single form rather they are found in phenyl-propanoids, Quinines, Flavonoids, Tannins and also may as in simple phenols.

Polyphenols or Polymeric combines with the acid molecule and forms the compounds like "Para-hydroxybenzoic acid", Vanillic acid , Pro-catechuic acid , Syringic acid, the Salicylic acid , Gallic acid and became responsible for theproduction of Lignin, Melanin, Flavolan, and Tannins in the most of the plant parts/tissues. The Basic phenol's, regarded as the monomer components for the poly phenol after breakage through favourable chemical reaction.

Glycosides

Glycosides are the compounds with a-glycone part (a non-sugar moiety) as well as glycone part (a glucose moiety) conjugated through α, β glucosidic bonds. They are classified into following types:

Anthracene glycosides: It contains a phenanthracene ring structure (aglycone part) attached to the glucose/sugar moiety,it is regarded as the distinctive feature of this type of glycosides. This glycosides are found in the plant Families like Ericacea, Euphorbiaceae,Leguminosae or Pea, Polygonacea, Rhamnoceae,Rubiaceae, etc.

Cardiac or Steroid glycosides: This glycosides consists of three 6-memberedand two 5-membered lactone rings & totally it contains C_{23} or C_{24} atoms in its structure. The 6-membered lactone rings are known as **Bufadienolides** & 5- membered lactone rings known as **Cardenolides**. The steriod glycosides are highly present in various plant families like *Euphorbiaceae, Cruciferae, Leguminoseae,Scrophulariaceae, Sterculiaceae,* etc.

Flavonoid glycosides: Glycosides contain Flavonoid ring (phenolic-hydroxy rings) which is attached to the glucose/sugar moiety hence these are known as flavonoid glycoside.They are present in some of the Dicot families like *Apiaceae , Compositae, Leguminosae/ Fabaceae, Polygonaceae, Rutaceae*, etc.

This type of glycosides are one of the class compounds found in phytochemistry which consists of 2 phenol rings that are fused with 3-C ring or propane unit. Thistype glycoside has different categories it can be represents to a aspect as supplementary (O) atom along to Anthocyanidins, Aurones Catechins, Chalcones, Flavanones, Flavonols, flavanonols, flavones, Iso-flavones, Leucoanthocyanidins, termed as the examples of heterocyclic rings.

Quinones are the components which gives aromatic odour during charred or burned, this quinones are situated mostly in the bark part of huge trees and they are highly pigmented. Usually quinones obtain from the precursors of anthraquinone or naphthoquinone , benzoquinone structures.

Alkaloids: Alkaloides are produced from phenylalanine, tyrosine and tryptophan precursors . These compounds have N-atom (1or >1) in the 5-membered ring moiety are virtually termed as alkaloids. The presence of heterogeneous group is responsible for therapeutic activity,which is highly potent. Alkaloids are divided into following various types.

Heterocyclic Alkaloid	Examples
Indole	Vincrsitine & Vinblastine
Imidazole	Pilocarpine & Iso-pilocarpine
Norlupinane	Lupanine &Cystine
Isoquinalone	Berberine, Papverine and Codeine
Pyrrolidine and Pyrrole	Hygrine
Pyridine and Piperidine	Lobeline and Arecoline
Purine	Theobromine and Caffeine
Quinalone	Quinidine and Quinine
Tropine	Hyoscine and Atropine
Steroidal Alkaloids	Conessine

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Protoalkaloids

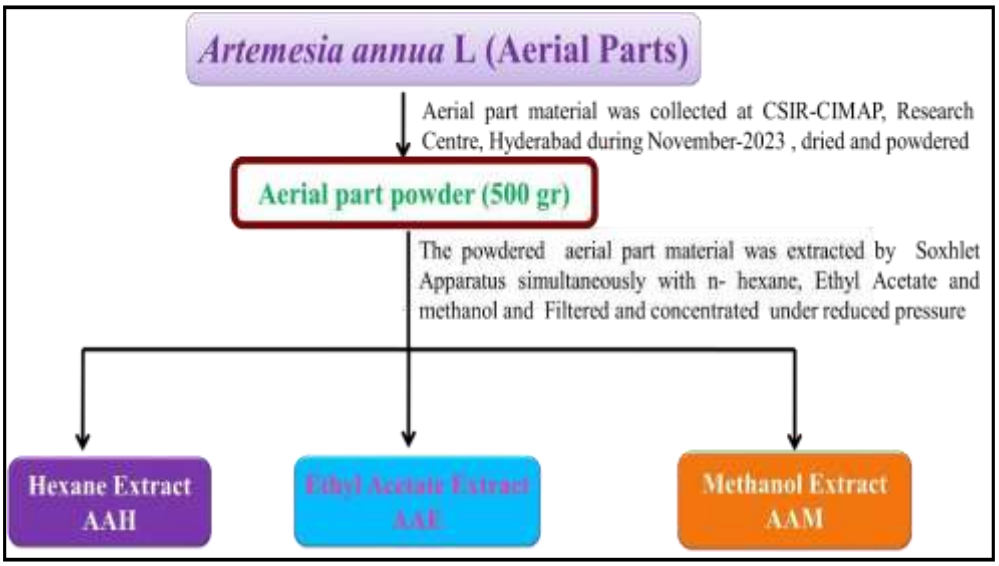
Alkyl amine (Aminoalkaloid) (Ex: Ephedrine).

II. MATERIAL & METHODS

- **COLLECTION OF PLANT**
- *A. annua aerial* parts was collected from the CSIR-CIMAP Research Centre in Boduppal, Hyderabad. **Dr. Venkat Ramana**, an Assistant Professor in the Department of Botany at Nizam College, Osmania University, Hyderabad, has verified the taxonomy of the plant. A voucher specimen (CIMAP-AA/23) was submitted in the CIMAP Research Center, Hyderabad".
- **Procedure for Soxhlet process**

The soxhlet apparatus was initially packed with cotton to prevent the entry of plant material into the siphon tube. Then the *A. annua* plant powder was packed into the long vertical glass cylindrical chamber of the Soxhlet extractor from large opening above it. The Soxhlet extractor was fixed to the neck of 5 L round bottom flask containing the solvents i.e., non polar and polar (nearly 1500-2000 mL). The condenser was connected to top open end of soxhlet. The solvent was continuously heated by reflux process on heating mantle. Solvent vapors after boiling moves into the condenser tube, condenses into a liquid droplet form after touching to the cool surface of condenser tubes falls down into the chamber holding the plant material. The surface of plant material gets slowly filled with the solvent returning from the condenser & side tube. When the Soxhlet chamber gets filled to required level, then the chamber gets emptied due sudden pressure fall and by the siphon tube side arm, solvent extract flows back into the solvent flask. This cycle was allowed to repeat for many times. The solvent extract from the R. B flask was collected, filtered and then concentrated using rotary evaporator. The percentages of the extracts were calculated by following formula.

Extractive yield (% W/W) = Extract weight / Plant material wt X 100



SECLUSION OF ACTIVE COMPONENTS FROM *A. ANNUA*

A.annua plant material was elicitated with simultaneously with Hex., EtOAc and MeOH by soxhlet apparatus. The EtOAc and MeOH extracts were refluxed with hexane. The hexane soluble portions were mixed with hexane extract. The obtained extract was subjected to C.C using silica gel as an adsorbent and the column was run successively with hexane and mixture of EtOAc in hexane.

- Column has covered (60cms * 3 cm) by sterilized cotton with the help of hex.
- 11 gms of elicitation have to be solubilized in $CHCl_3$ and poured against the absorbent and made it slurry then loaded on to the prepared column.
- Initially the column was eluted with hexane about 500 mL of each fraction contaminants were removed .Then MP about 5% of EtOAc with hexane was prepared.

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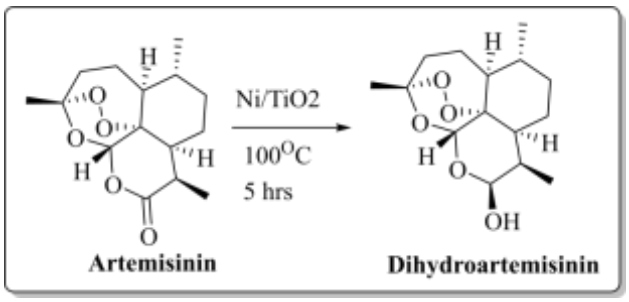
- Initial 5 fractions consists impurities like fatty material and other dyes. After 5 fractions identicalfractions are mixed and concentrated and crystalized with hexane yielded marker compound
- **Artemisinin (1.5 g)** and monitored in TLC using mobile phase 10 % EtOAc in hexane system.
- Then the polarity was increased up to 10 % EtOAc in hexane.
- First 5 similar fractions are mixed and concentrated and crystalized with hexane yielded **Artemisinin (0.3 g)**. Same mobile phase was continued in the column, compound **Artemisinin** slightly appeared in TLC but no content obtained on concentration. Hence the polarity was increased up to 15 % EtOAc in hexane.
- In 15% EtOAc in hexane fractions did not shown marker compound **Artemisinin** spot in TLC

CONVERTION OF ARTEMSININ TO DIHYDROARTEMSININ

"DHA compound was synthesized from artemisinin by Ni/TiO2 catalyst in methanol by previously reported method (Komuraiah er al., 2021)".

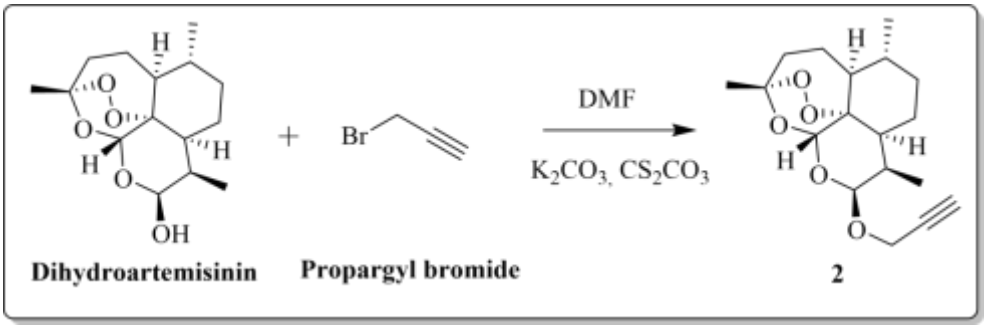


SYNTHESIS OF NOVEL DIHYDROARTEMSININ 1,2,3-TRIAZOLES

Dihydroartemsinin derivatives were produced mostly by altering the hydroxyl moiety. We had succeeded in synthesizing the series of novel Dihydroartemisinin-1,2,3-triazoles by considering the pharmacological properties.

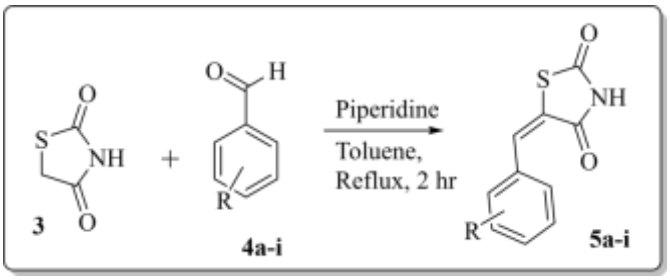
Step-I: Dihydroartemsinin Propargylation :

Propargylated DHA was synthesized by previously reported method (Devender et al., 2021).



Step II: Synthesis of compound 5a-i (Knowengal Condunsation)

Components about 5a-I had produced which was previously reported method (Yakaiah er al.,2012).



Step III: Benzylation of 5a-i

Components by benzylation **5a-i** were executed through Components 5a-I was prepared using previously reported method (Yakaiah er al., 2012).

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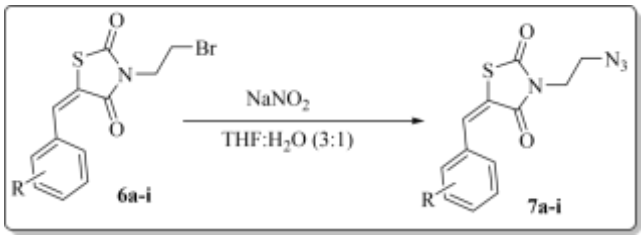
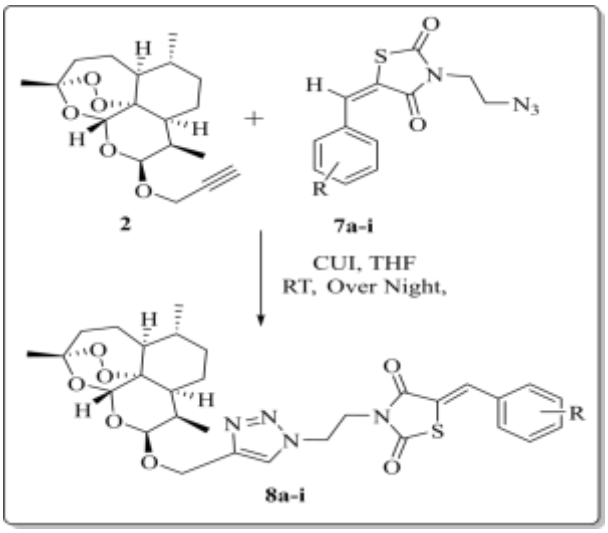
Step-IV: Synthesis of azides

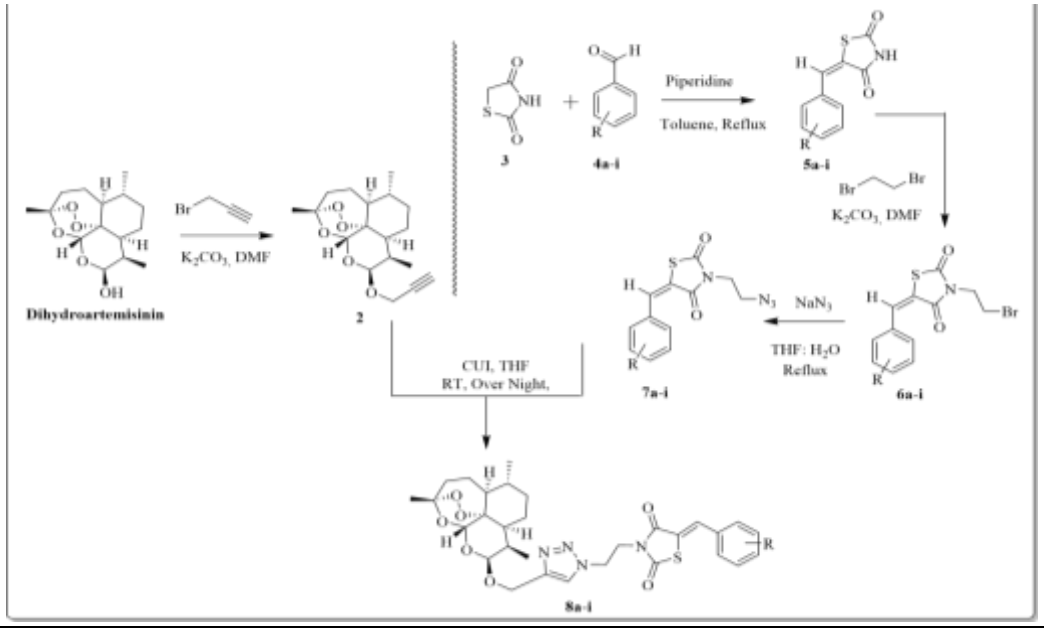
Compounds **7a-i** (1 eq.) was synthesized based on the previous reports (Yakaiah et al. 2015)



Step-V: Synthesis of Novel Dihydroartemsinin 1, 2, 3 Triazoles

Final derivatives were synthesized based on the previous reports (Yakaiah et al. 2015)





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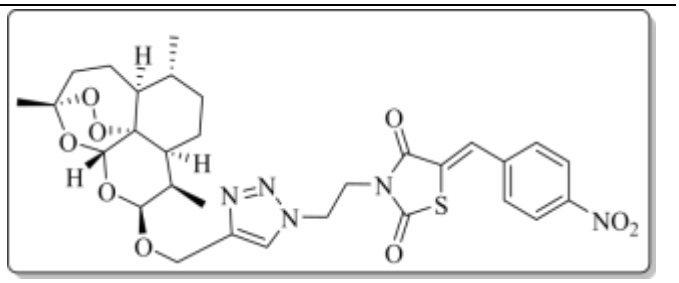
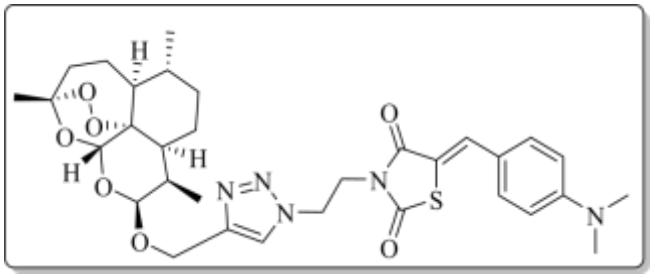
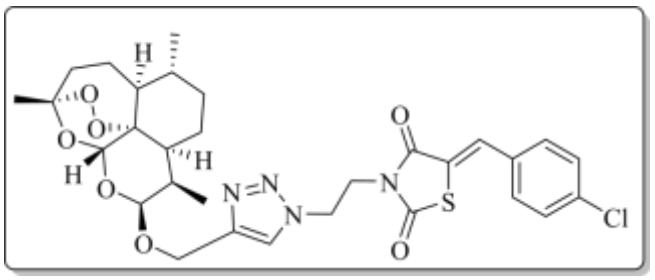
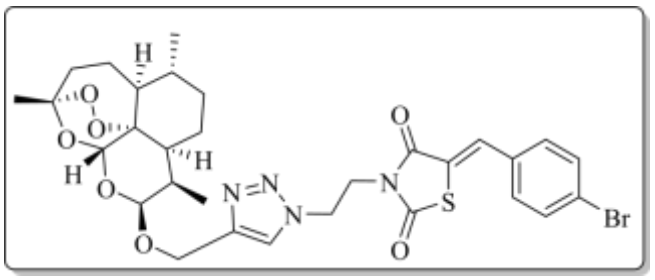
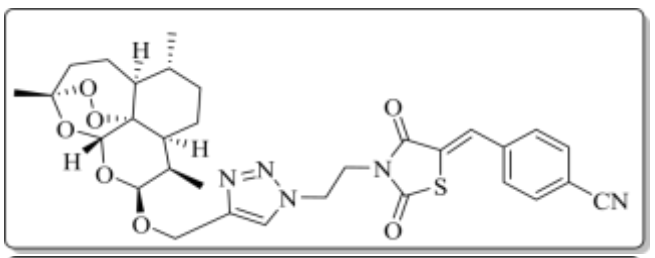
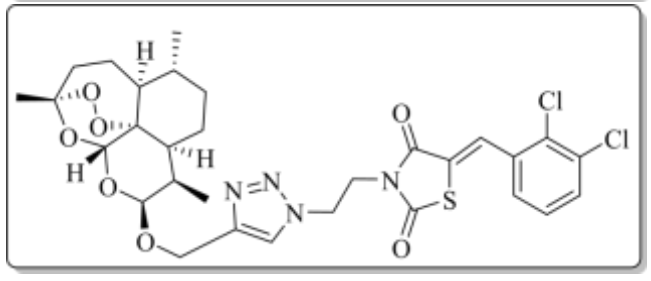
Synthesis of Dihydroartemsinin -1,2,3-triazoles.

Where R =

Code	Structure
8a	
8b	
8c	
8d	
8e	
8f	

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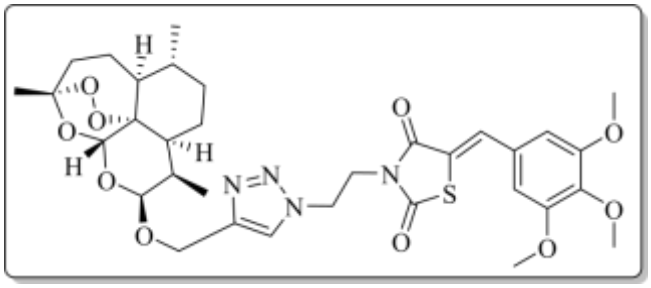
8g



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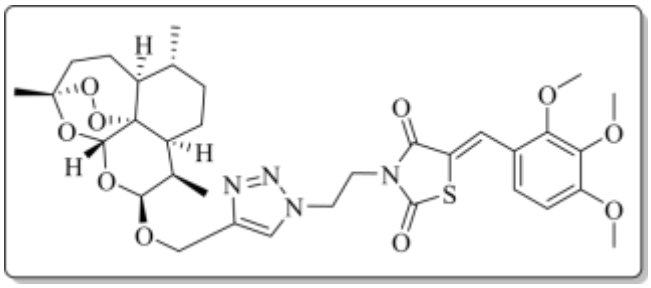
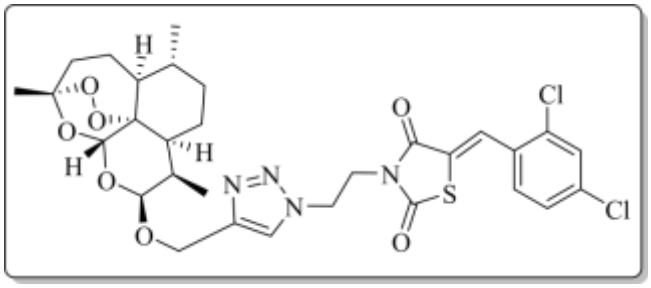


8i



All the artificial byproducts of chemical configuration have to be illuminated by considering various spectrum analysis (Proton NMR, Carbon-13 NMR, Mass spectroscopy and Infra-Red Spectroscometry).

ACTIVITY SCREENING

The semi-synthesized derivatives of Dihydroartemsinin -1,2,3-triazoles had capitulated to study the pharmacological activities - Anti-Cancer, Anti-Diabetic, Anti-Microbial etc.

III. RESULTS AND DISCUSSIONS

A. annua aboveground plant parts powder was subjected to successive solvent extraction with different solvents.

S. No	Plant Part	Extract	extrication Proceudre	Pigment	Weight (gms)	%Yield (%W/W)
1.	Aerial Parts	Hexane (AA-H)	Soxhlet	Light green semisolid	11	2.2
2.		Ethyl acetate (AA-E)	Soxhlet	Dark green semisolid	35	7.0
4.		Methanol (APAM-C)	Soxhlet	Dark brownish red semisolid	30	6.0

PRELIMINARY PHYTOCHEMICAL SCREENING (PPS)

PPS of the extracts of the plant *A. annua* aerial parts has shown following results

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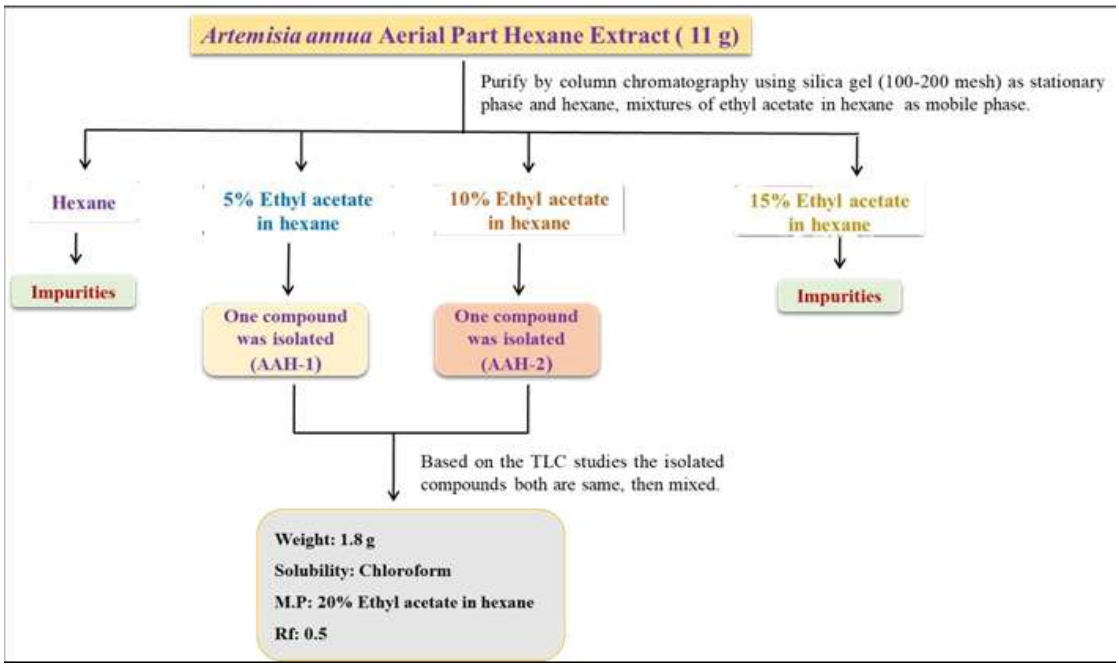
S. No	Constituents	AA-H	AA-E	AA-M
1.	Alkaloids	--	--	--
2.	Saponins	--	+	+
3.	Terpenoids	+	++	++
4.	Steroids	+	+	+
5.	Glycosides	--	++	++
6.	Fats and oils	++	--	+
7.	Tannins	--	+	++
8.	Phenols	--	++	++
9.	Flavonoids	--	++	++
10.	Amino acids and proteins	--	+	+
11.	Carbohydrates	--	++	++

Based on the PPS of the extracts MeOH extract contain abundant phytochemicals like terpenoids, glycosides, tannins, phenols, flavonoids etc.

MARKER COMPOUND SECLUSION

The plant material was extracted with hex using soxhlet apparatus. The obtained extract was filtered and concentrated. The obtained extract was purified by C.C eluted with hexane and mixtures of Et.OAc in hexane as mobile phase to marker compound Artemesinin in pure form.



CONVERTION OF ARTEMSININ TO DIHYDROARTEMSININ

SYNTHESIS OF NOVEL DIHYDROARTEMISININ 1,2,3-TRIAZOLES.

A series of compounds (**8a–i**) are synthesized in four steps. In the 1st, DHA 1 treated with propargyl bromide in the presence of K_2CO_3 as catalyst and N, N-DMF as solvent yielded compound **2**. In the second step, A mixture of aldehydes **4a-i** reacted with 2,4-thiazolidinedione in the presence of piperidine as a catalyst at reflux

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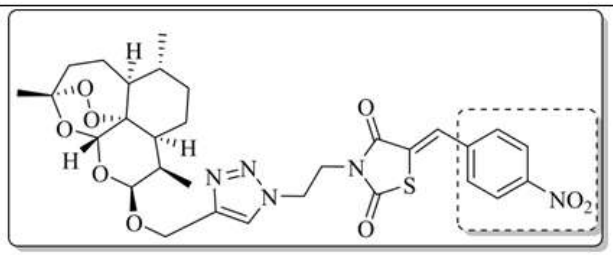
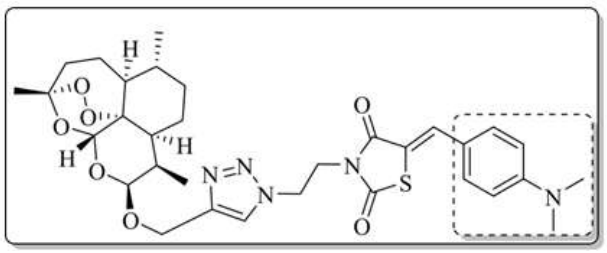
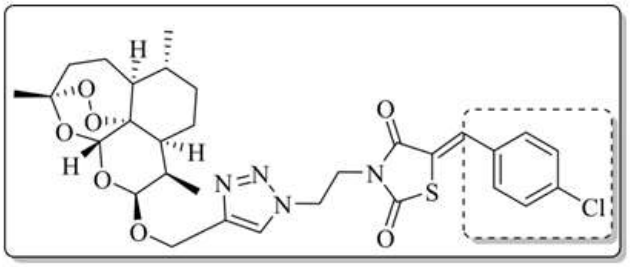
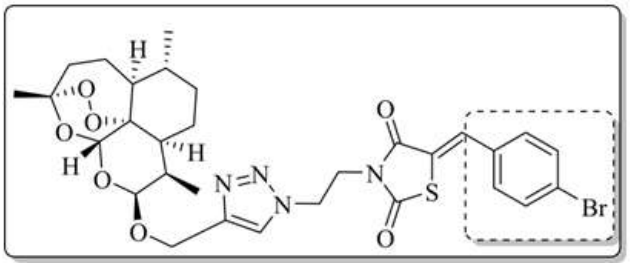
condition for 4 hr to give Knowengal condunsation products **5a-i**. In the third step compounds **5a-i** were reacted with 1,2- Dibromoethane in the presence of K_2CO_3 at RT for 5 hr gave brominated Knowengal condensation products **6a-i.** In the fourth step, series of azides were synthesized from respective brominated Knowengal condensation products **6a-i** using NaN_3 and THF: Water. All the analogues were characterized by Infra Red, proton and Carbon-13 NMR & mass. Proton NMR component **2**, the OH-peak had absent where delta is 4.20 parts per million that indicates OH- moiety linked with propargyl moiety & then confirmed based on presence of methyl groups where delta is 3.18 parts per million. Comparably, in ^{13}C NMR presence of apex vibrations where delta is 44.62, 76.80 & 82.40 parts per million indicate that 1-propyn-3-ol moiety which was situated inside the molecule.

But, Proton Nuclear Magnetic Resonance components of 6f vibrations triplet visible where delta is 4.21 and 3.63parts per million that illustrates 1,2 dibromoethane which were paired using NH proton & then finalized through Carbon-13 Nuclear Magnetic Resonance, -CH_2- peaks visualized where delta is 26.42 and 42.83 ppm. In the ^1H-NMR the presence of characteristic peaks of 8f singlet at δ 8.05 ppm corresponding to CH=N of 1,2,3-triaz and at δ 5.06 ppm and 4.08 ppm were due to two methylene protons, also which further confirmed by in ^{13}C-NMR at δ 5.06 ppm 128.08 and 43.40 and 62.00 ppm respectively. The dihydroartemesinin protons appear between δ 0.85 to 2.5 ppm.

S. No	Component	Chemical Structure	Yield (%)
01	**8a**		72
02	**8b**		81
03	**8c**		75
04	**8d**		75

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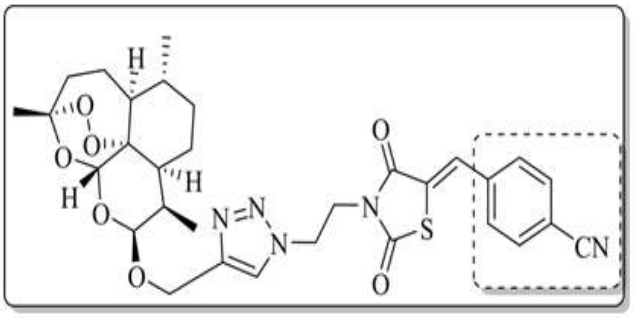
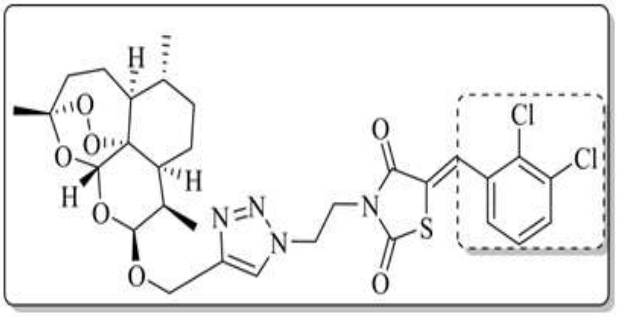
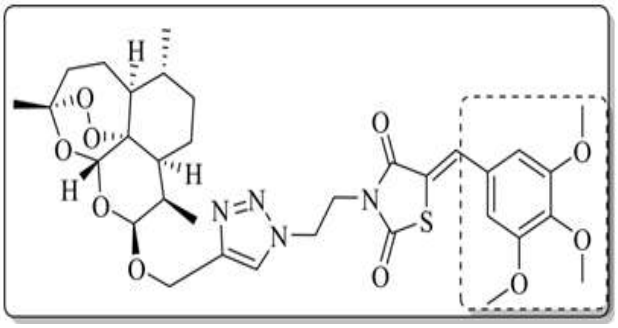
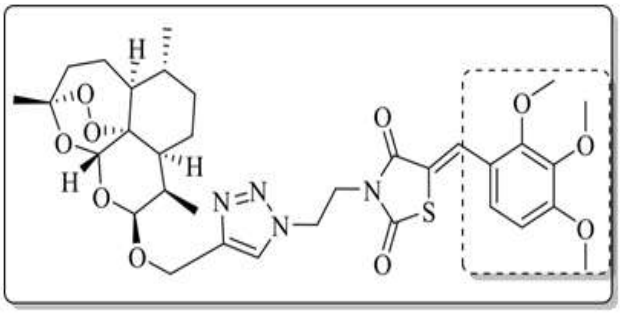
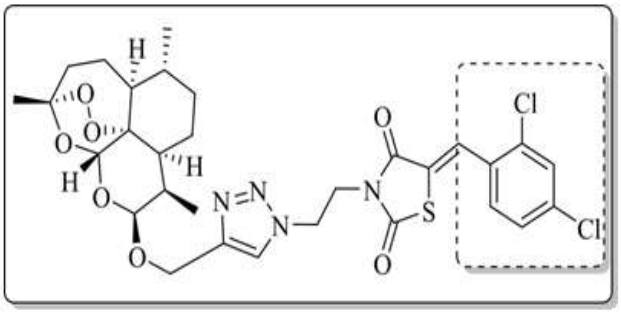
05	8e		72
06	8f		65
07	8g		80
08	8h		74
09	8i		72

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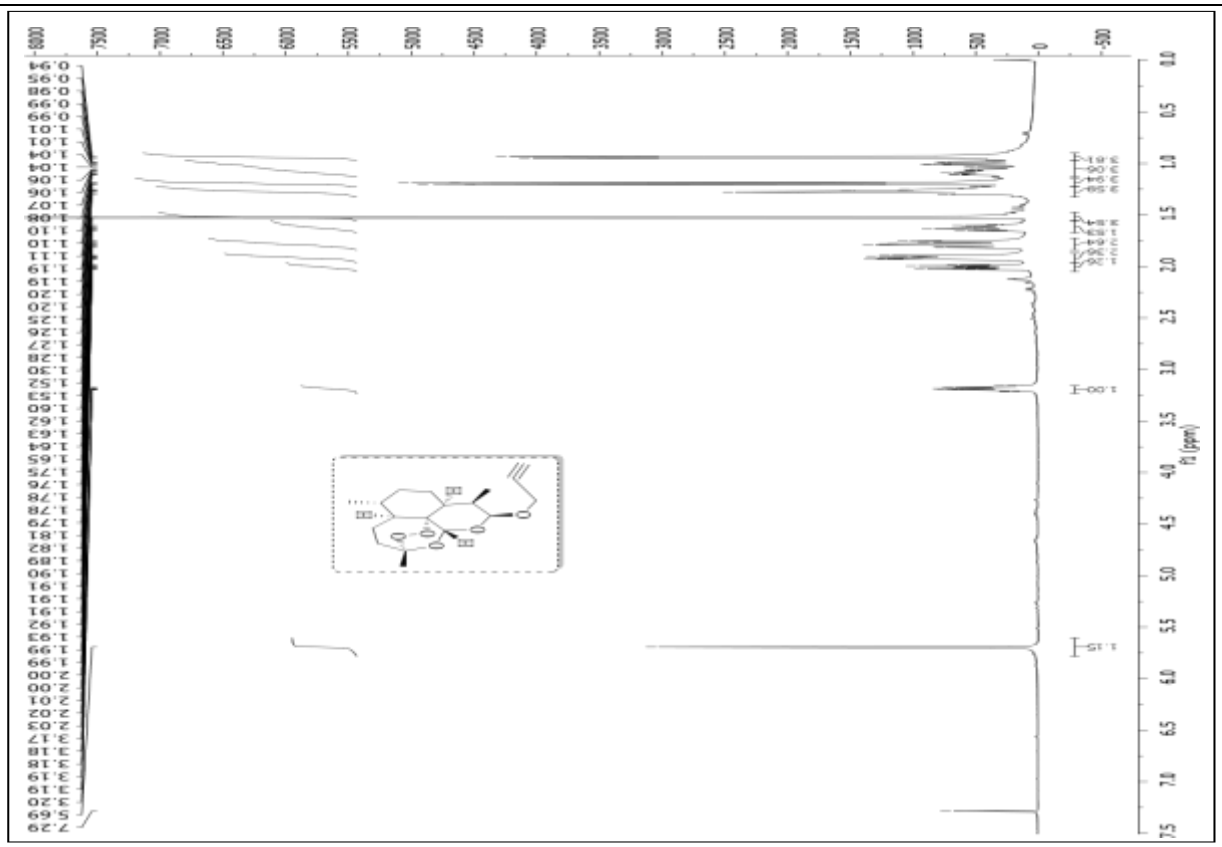

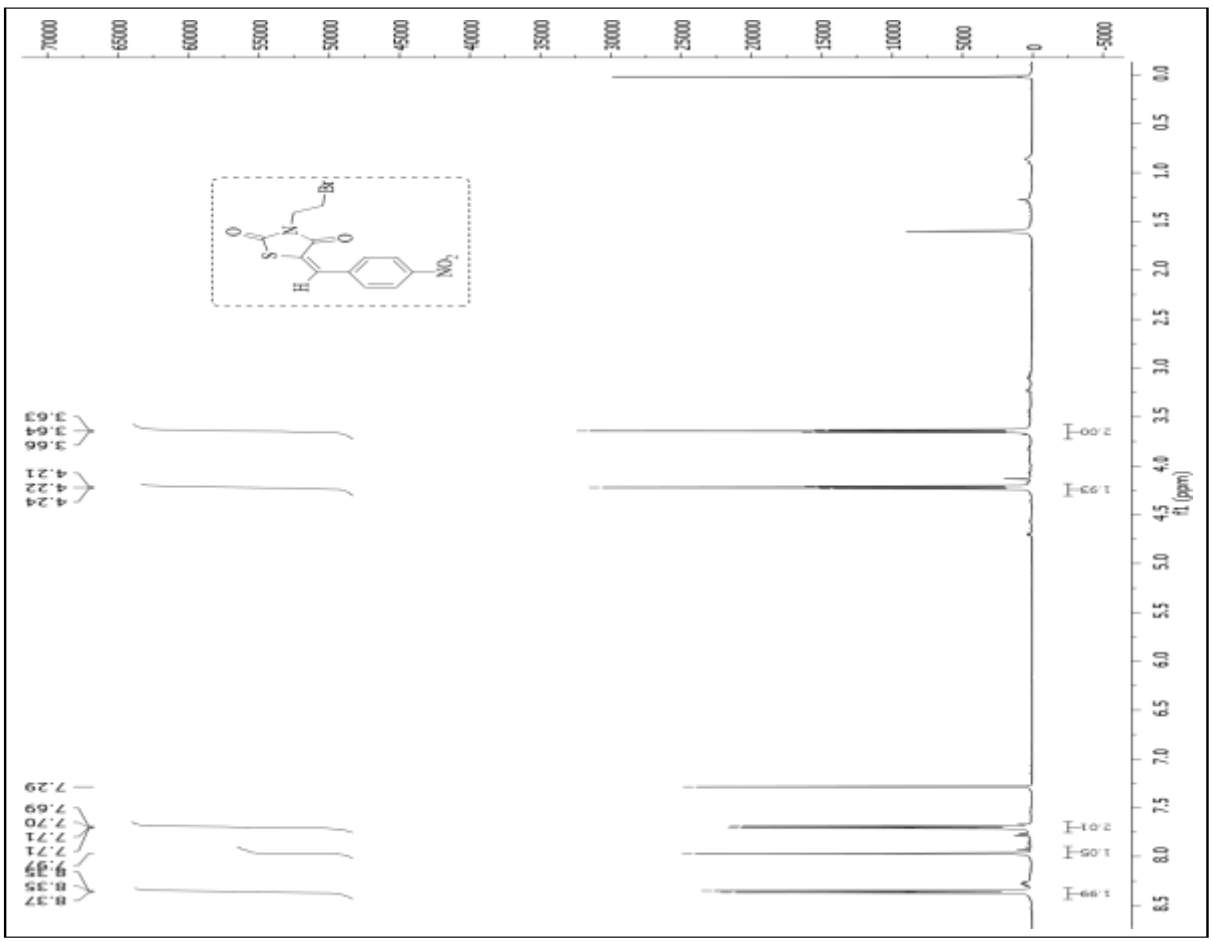

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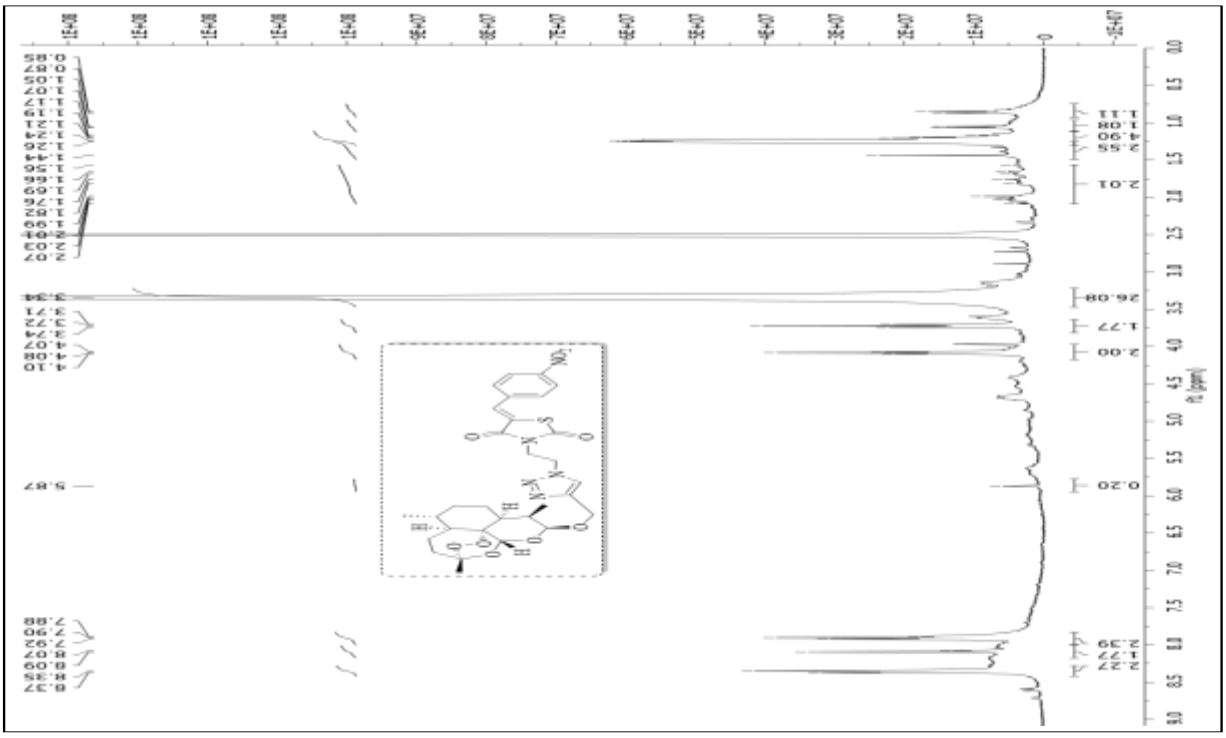

IV. CONCLUSION

The present thesis describes ***"Isolation of Anti-Malerial Compounds from Artemesia Annua L and Semi-Synthesis of Novel Dihydroartemesinin Derivatives"*.**

The aerial part material was extracted with hexane, EtOAc and MeOH by soxhlet apparatus. The percentage yields of hexane extract (**AH-H**; 2.2%), EtOAc (**AA-E**: 7.0%) and MeOH extract (**AA-M**; 6.0 percentage) produced with the help of dried content of A. annua. The yield % for EtOAc product were high comparing with other contents.

Then hexane extracts and hexane soluble portions of EtOAc and MeOH extracts were subjected to C.C for the isolation of marker compound. The marker compounds **AAH-1** and **AAH-2** were isolated (both are same) from hexane product & %yield of illustrated components from warm product/decoction discovered about 1.8 g.

The structures of the isolated marker compound was identified by as a **Artemesinin** with reference standard by TLC studies. The isolated marker compound **Artemesinin** was converted in to DHA by reduction with Ni/TiO2 at reflux condition. The obtained Dihydroartemesinin was used for semi-synthesis of DHA 1,2,3-triazoles by click chemistry. Analogues (**8a–i)** are synthesized in four steps. In the 1st step, DHA one treated using propargyl Br in the sight of Pot. Carbonate and Nitro, N-DMF as solvent yielded Propargylated DHA **2**. In the second step, mixture of aldehydes **4a-i** reacted with 2,4-thiazolidinedione in the presence of piperidine as a catalyst at reflux condition for 4 hr to give Knowengal condunsation products **5a-i**. In the third step the products **5a-i** were reacted with 1,2-Dibromoethane in the presence of K_2CO_3 at RT for 5 hr gave brominated Knowengal condunsation products **6a-i.** In the fourth step, series of azides were synthesized from respective brominated products **6a-i** using NaN_3 and THF: Water.

V. REFERENCES

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