ABSTRACT

(As required under clause 13 of ordinance VI of the University of Delhi)

Abstract of the thesis entitled: “Synthesis of Triazolylated Nucleosides, Coumarins and Coumarinyldihydropyrimidinones and Selective Biocatalytic Acylation & Antifungal Activity Studies on Coumarins”

The thesis is divided into three chapters, i.e. Chapter I, Chapter II and Chapter III. Chapter II is further divided into two Sections, i.e. Section A and Section B. A brief account of each chapter is given below:

The work presented in Chapter I entitled “Synthesis of 3’-substituted Triazolynucleosides” describes the synthesis of 1, 2, 3-triazolynucleosides via click reaction of 3’-azidonucleoside and coumarin, aryl and alkyl functionalized alkynes.

The copper (I)-catalyzed Huisgen-Sharpless-Meldal click chemistry has gained significant importance because of its wide range of applications in various fields of drug discovery, bioconjugation and material or surface science. A very promising area for the preparation of new bioconjugates is the synthesis of azidonucleosides. In order to discover new derivatives potentially endowed with biological activity, the copper-catalyzed azide/alkyne 1,3-dipolar cycloaddition reaction has also been applied to the functionalization of nucleoside sugar and base moieties. And coumarin derivatives are widely used as fluorescent probes, labels and pigments, laser dyes and signalling units in sensors. They are also attractive molecules due to their extended spectral range, high emission quantum yields, photo stability.

In the present work, we achieved the synthesis of a series of coumarin-, aryl- and alkyl- conjugated 1,2,3-triazolynucleosides via click reaction. To synthesize the target compounds, the first key substrate, 3’-deoxy-3’-azido-5-methyluridine was prepared in seven steps from readily available D-xylose. First of all, D-xylose was selectively protected as a monoketal, 1,2-O-isopropylidene-α-D-xylofuranose
followed by selective protection at primary hydroxyl using benzyol chloride and pyridine and then 3'-hydroxy group was converted to 1,2-\textit{O}-isopropylidene-5-\textit{O}-benzoyl-3-\textit{O}-trifluoromethanesulfonyl-\textit{a}-D-xylofuranose using trifluoromethanesulfonic anhydride. It was then subsequently converted to a 3-azido-1,2-\textit{O}-isopropylidene-5-\textit{O}-benzoyl-3-deoxy-\textit{a}-D-xylofuranose with sodium azide, which was further converted into 3-azido-1,2-di-\textit{O}-acetyl-5-\textit{O}-benzoyl-3-deoxy-\textit{B}-D-ribofuranose using a mixture of acetic acid, acetic anhydride in pyridine. The compound so obtained was coupled with thymine as base by using BSA and trimethylsilyl triflate. Finally, deacylation was done to afford 3'-deoxy-3'-azido-5-methyluridine. The second key precursor coumarin and naphthalene derivatives with terminal alkynes were prepared \textit{via} an established method from different coumarins and naphthalenes by the use of propargyl bromide, K$_2$CO$_3$ in acetone. Then the mild reaction conditions and high fidelity of Cu (I)-catalysed process allowed the 1,3-dipolar cycloaddition reaction of 3'-deoxy-3'-azido-5-methyluridine with alkynes phenylacetylene/ propargyl alcohol/ 5-chloro-1-pentyne/ propargyloxycoumarins and propargyloxy naphthyl by using 0.15 molar equiv of CuI in solution of THF: H$_2$O:EtOH (1:1:1) mixture at 60 °C affording 3'-deoxy-3'-(4-substituted-1,2,3-triazol-1-yl)-5-methyluridine. The structures of all the synthesized compounds were unambiguously established by analysis of their spectral data (\textit{H} NMR, \textit{C} NMR, IR, HRMS spectra).

The work presented in \textbf{Chapter II: Section A} entitled \textit{“Synthesis and Antifungal Activity of Novel Azido & 1,2,3-triazole containing coumarins”} describes the synthesis and antifungal activity of azido and triazole containing coumarin derivatives.

The alarming rates of the growing emergence of antifungal resistance are major concerns to the public health and scientific communities worldwide, especially in the field of multidrug resistant bacteria and fungi. These trends have emphasized the urgent need for new, more effective, less toxic and safe antifungal agents and the development of structurally new classes of antifungal with novel mechanisms of action as well as structural modifications to improve both their binding affinity and their spectrum of activity. One such strategy that has been pursued in recent years
with increasing significance employs a combination of two different active fragments in one molecule. With this strategy, various drug moieties have been designed to bind independently to different biological targets to produce beneficial effects. The 1,2,3-triazole scaffold, a small molecular heterocyclic sub-structure, is very important in the field of medicinal chemistry and has received much attention in the last few decades due to its chemotherapeutic value. Coumarins (Benzopyran-2-ones) form an elite class of compounds, which occupy a special role in nature and have been isolated both from synthetic and natural sources. It was found to be crucial for a variety of pharmacological effects such as inhibition of platelet aggregation, anti-inflammatory, anti-viral, anti-HIV, anti-coagulant, anti-oxidant, anti-bacterial, anti-tubercular, anti-carcinogenic and anti-fungal.

In the present work, we have synthesized a new series of azido and triazolyl derivatives containing a coumarin backbone via Cu (I)-catalyzed click reaction and investigated their antifungal potential using Aspergillus as model pathogens. These compounds contain different alkyl chain at the C-3 position of the coumarin ring and different aryl ethers at the C-4 position of 1,2,3, triazole ring. The synthesis of desired product was achieved starting from C-3 alkyl coumarins. This was synthesized via Pechmann condensation of resorcinol and C-2 alkylated β-keto ester. These coumarins were coupled with epichlorohydrin followed by epoxide ring opening with sodium azide to afford 7-(3-azido-2-hydroxypropoxy)-3-alkyl-4-methylcoumarin. Then Cu (I)-catalyzed process allowed the 1,3-dipolar cycloaddition reaction of 7-(3-azido-2-hydroxypropoxy)-3-alkyl-4-methylcoumarin with alkynes (phenyl acetylene, pent-4-ynoic acid, 4-chloropentyne and propargyl alcohol) and with propargyl aryl ethers in the presence of catalytic amount of copper sulphate and sodium ascorbate in t-BuOH/H₂O/THF at 50 °C to afford 7-(3-(4-substituted-1,2,3-triazol-1-yl)-2-hydroxypropoxy)-3-alkyl-4-methylcoumarins.

Thus, a series of fifty two azido and 1, 2, 3-triazole containing coumarins have been synthesized and evaluated as antifungal agents. And the results clearly show that among all the tested azido-coumarins and 1,2,3-triazole containing coumarins, 7-(3-azido-2-hydroxypropoxy)-3-ethyl-4-methylcoumarin was the most potent compound and inhibited even at low concentration of 3.91 μg/disc in disc diffusion (DDA) and 15.62 μg/ml in microbroth dilution assays (DDA) in case of *A. fumigatus*. 7-(2-Hydroxy-3-(4-hydroxymethyl)-1,2,3-triazol-1-yl)propoxy)-3-hexyl-4-
methylcoumarin was the next most active compound and showed appreciable activity against all three fungal strains such as A. fumigates, A. niger and A. flavus. Some compounds exhibited moderate to good antifungal activity. From the analysis of results, it was revealed that antifungal activity decreases considerably with the increase in the chain length at the C-3 position of coumarin.

The work presented in Chapter II: Section B entitled “Enzymatic Stereoselective Acylation Studies on Novel Azido and 1, 2, 3-Triazole Containing Coumarins” describes the enantioselective acylation studies on biologically active azido and 1, 2, 3-triazole containing coumarins.

Since several years, enzymes are being recognized as efficient catalyst for many of the stereo-specific and region-selective reactions. The potential of enzymes is well recognized for selective acylation /deacylation of different functional groups of similar reactivity present in the molecule. Some of the lipases have been found selective for acylation/deacylation of hydroxyl group(s). This stimulating background and our own interest in the lipase-mediated chemical transformations prompted us to explore the possibility of lipase-mediated selective acylation studies on antifungal racemic azidocoumarins and 1,2,3-triazolylcoumarins synthesized in Chapter II Section A. In this chapter, we have done enzymatic resolution of biologically active azidocoumarins and 1,2,3-triazolylcoumarins by enzymatic acylation reaction using CRL (Candida rugosa) as biocatalyst.

Different lipases, i.e. Candida antarctica lipase-B (CAL-B), Theremomyces lanuginosous lipase immobilized on silica (Lipozyme TL IM) and porcine pancreatic lipase (PPL) were screened for enantioselective acylation of racemic azidocoumarins and triazole containing coumarins in six sets of organic solvents, i.e. diisopropylether (DIPE), toluene, tetrahydrofuran (THF), dioxane, acetonitrile (CH$_3$CN) and acetone using vinyl acetate as acylating agent for acylation at 50 °C and at 200 rpm in an incubator shaker. Initial screening of reactions was done at small scale and reactions were monitored using TLC. Lipase CRL in toluene at 50 °C was found to be the best combination for carrying out the enantioselective acylation of azidocoumarins and triazolylated coumarins. Optical rotations of unreacted laevorotatory hydroxyl substrates and the corresponding dextrorotatory hydroxy azidocoumarins and
triazolylated coumarins obtained by chemical hydrolysis of enzymatically acylated dextrorotatory azidocoumarins and 1,2,3-triazolylated coumarin substrates were found to be comparable. This shows the selectivity of CRL for the acylation of particularly one enantiomer in the racemic azidocoumarins and 1,2,3-triazolylated coumarins.

The work presented in Chapter III: entitled “Synthesis of Novel Coumarinyldihydropyrimidinones and their N-acylates” describes the synthesis of coumarinyldihydropyrimidinones and their N-acylates.

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores may lead to the compounds with interesting biological profiles. Heterocyclic compounds, viz. coumarin and their derivatives, and dihydropyrimidinones are well studied compounds and attract attention of scientists by their diverse biological properties, like anticoagulant, antifungal, antibiotics, antimicrobial, antiviral, antioxidant, anticancer, anti-inflammatory, etc. Encouraged by these results shown by coumarins and dihydropyrimidinones, we have synthesized coumarinyldihydropyrimidinones and their N-acylates. It is expected that this hybrid molecule may exhibit synergistic effects.

In the present work, we have synthesized a series of new coumarin based dihydropyrimidinones. Coumarins were synthesized following the Pechmann condensation followed by methylation, oxidation at C-4 position yielding methylated 4-formylcoumarins. Dihydropyrimidinones were synthesized by acid catalyzed Biginelli reaction of 4-formylcoumarins with urea and appropriate β-ketoester by use of sulphuric acid as catalyst in ethanol yielding the desired coumarinyldihydropyrimidinones in quantitative yields. Thus CDHPMs having variation at the C-6 and at the ester linkage of the DHPM ring and C-5, C-7 and C-8 position of coumarin ring were synthesized. Further, 7,8-dimethoxy CDHPMs were acylated at N-3 position with acid anhydride (acetic-, propanoic-, butanoic-, pentanoic-, hexanoic and benzoic anhydride) in DCM at room temperature using 4-N,N-dimethylanminopyridine (DMAP) as a catalyst to afford N-acylated derivatives. Hence, a series of thirty seven different coumarinyldihydropyrimidinones and their N-acylates were synthesized having different ester chain and acyloxy chain. The
structures of all synthesized thirty seven compounds were unambiguously established on the basis of their spectral data ($^1$H, $^{13}$C NMR, IR and HRMS) analysis.

The work presented in the thesis entitled “Synthesis of Triazolylated Nucleosides, Coumarins and Coumarinyldihydropyrimidinones and Selective Biocatalytic Acylation & Antifungal Activity Studies on Coumarins” by Ms Anu Arya for the award of the degree of Doctor of Philosophy of the University of Delhi, has been carried out in the Laboratory of the Department of Chemistry, University of Delhi, under the supervision of Professor Ashok K Prasad. The work is original and has not been submitted in part or in full, for any other Diploma or Degree of this or any other University.

The extent of information derived from the existing literature has been indicated in the body of the thesis at appropriate places giving the source(s) of information.

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