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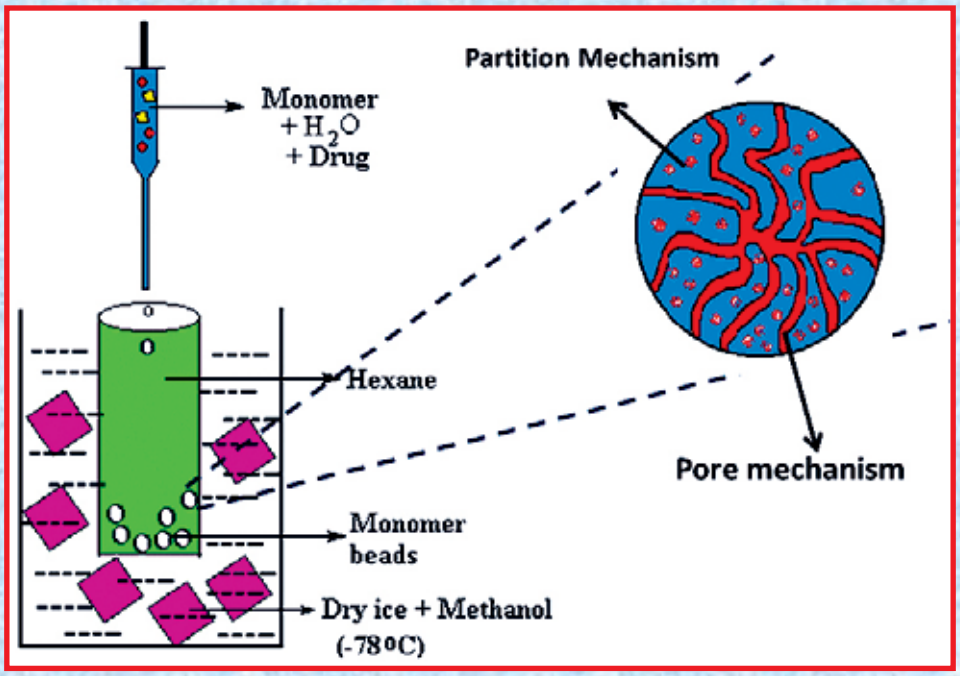


**Special Issue on**

## Materials for Healthcare

SOCIETY FOR MATERIALS CHEMISTRY

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Society for Materials Chemistry was mooted in 2007 with following aims and objectives:

(a) to help the advancement, dissemination and application of the knowledge in the field of materials chemistry,

(b) to promote active interaction among all material scientists, bodies, institutions and industries interested in achieving the advancement, dissemination and application of the knowledge of materials chemistry

(c) to disseminate information in the field of materials chemistry by publication of bulletins, reports, newsletters, journals

(d) to provide a common platform to young researchers and active scientists by arranging seminars, lectures, workshops, conferences on current research topics in the area of materials chemistry,

(e) to provide financial and other assistance to needy deserving researchers for participation to present their work in symposia, conference, etc.

(f) to provide an incentive by way of cash awards to researchers for best thesis, best paper published in journal/national/international conferences for the advancement of materials chemistry,

(g) to undertake and execute all other acts as mentioned in the constitution of SMC.

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SOCIETY FOR MATERIALS CHEMISTRY

# SMC Bulletin

**Vol. 9** **No. 3** **December 2018**

**Guest Editor**

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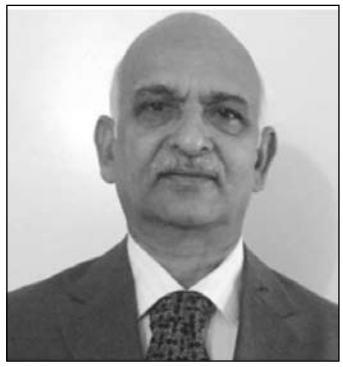
*Front cover shows radiation induced synthesis of porous polymeric matrices of (2-HEMA+EGDMA+NVP/MMA).*

# *Guest Editorial*



**Dr. G.A. Rama Rao**

Every era poses unique challenges to healthcare organizations, and the beginning of the twenty-first century has been no different. The mindset and attitudes of people about many social issues are chiefly responsible for creating many emotional health issues. Today there is an unprecedented attention on the quality of health services considering the eruption of many new diseases that are resistant to various medicines. A healthy life style is an answer to many emotional based health problems. However the materialistic

However, the current treatment procedures rely heavily on conventional chemical agents. Hence the need to develop various new pharmaceutical formulations has become the genesis of research. Hyderabad city has come to be known as a pharmaceutical hub by providing a major share of research and production of export grade active pharmaceutical ingredients (API).

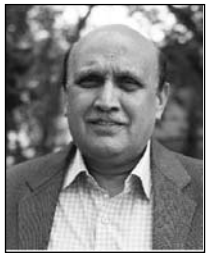
School of Studies in GITAM University, Hyderabad has organized the symposium on "Materials in Healthcare" and the title has drawn wide attention amongst the chemistry fraternity because a major quantum of research is being devoted to organic synthesis, characterization and biological testing for various applications in healthcare. With the advent of computers, the search has manifested in the designing of new molecules and discerns novel schemes of synthesis.

I am thankful to the managing committee of SMC for helping us to conduct the symposium and also for assigning me with the responsibility of collecting and editing of a few articles to be published in SMC bulleting.

The authors are well known in the chosen fields and are active in the pursuit of synthesizing and evaluating the biological activity of several materials that have promise in medicine. The articles cover the gamut of synthesizing of materials that find application in treatment of diseases, particularly cancer, protecting DNA from oxidative stress, role of intelligent polymer nanostructures and a few new nanocatalysts in designing new synthetic schemes. Another article deals with the efficacy of radiation effects on polymeric materials that find application in Healthcare.

(Guest Editor)

# From the desks of the President and Secretary



**Dr. V. K. Jain**
*President*

**Dr. P. A. Hassan**
*Secretary*

Dear Fellow Members and Readers,

It is our pleasure to introduce to you this issue of SMC Bulletin that focuses on healthcare materials. SMC, in association with Gitam University Hyderabad, successfully conducted a symposium on materials for healthcare technologies. We are happy to let you know that the symposium was attended by a large number of students, researchers and faculty membersfrom both academia and industry. The challenges encountered by healthcare professionals in terms of materials' development is manyfold. The above symposium gave an opportunity to professionals working in different areas to discuss recent developments in the area of materials for health care. This became the platform to assimilate a few articles from the invited speakersin the symposium.

New strategies for targeting drugs to breast cancer, plant derived anti-genotoxic molecules and its linkages to traditional Indian medicine, stimuli sensitive and polymer based drug delivery systems, the role of radiation in processing of polymer based healthcare materials, development of new formulations and nano-structured materials, etc. are important areas in healthcare technologies. This has been discussed in detail in this issue.

We thank the guest editor, Prof. G. Rama Rao for his painstaking efforts in compiling this issue. Thank are also due to all contributing authors for their timely submission of articles. We are confident that this issue will enrich our readers with the recent trends in healthcare materials. We look forward to continued support from our fellow members and readers.

# CONTENTS

# Large extracellular loop of CD151 is a novel therapeutic target for metastatic triple negative breast cancer

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**Abstract**

CD151, an important member of tetraspanin superfamily, regulates diverse regulatory functions in cancer progression through metastatic process. The current article presents critical analysis on recent insights on role of CD151 in breast cancer metastasis. The elevated expression of CD151 has been associated with aggressive nature, large tumor size, high nodal stage, advanced stage and absence of ER, PR and HER-2 in breast cancer. Experimental settings in various models have underlined the role of CD151 and its partner proteins in regulation of metastatic behavior of breast cancers. Studies by targeting via RNA interference have validated CD151 as a novel class of molecular target for triple negative breast cancer. Recently, another evidence has emerged that CD151 is also involve in drug resistance. Subsequent studies have demonstrated that small molecule inhibitors specific to various targets sensitized CD151 silenced cancer cells. Large extracellular domain of CD151 is receiving much attention in the recent past, because it is simultaneously affecting signaling as well as different cellular mechanisms associate with metastasis and drug resistance. Therefore, CD151 can be sensibly projected as a new therapeutic target of breast cancers.

**Key Words**: Breast cancer, CD151, drug resistance, metastasis and RNA interference.

## 1. Introduction

CD151, a four-transmembrane-spanning protein belongs to tetraspanin family, associate with regulation of both cellular and signaling mechanisms [1–5]. CD151 serve as scaffold for cell surface proteins and recruit signaling molecules [6]. This property makes CD151 to function differently in various cellular functions conjoin with cancer disease and normal physiology. Normally, CD151 is expressed at basal levels on cell surface, but overexpressed on cancer cells [7]. It promotes multiple cancer stages [8–10]. Contemporary studies have highlighted CD151 as a novel molecular target for cancer therapy [11]. Originally, CD151 was demonstrated on surface platelet/ leukocytesas a glycoprotein, subsequently on wide variety of tissues and cancer cell types [12]. Well established cancer databases showed aberrant expression of CD151 in breast cancer tissues, subtypes and cancer cell lines. These databases predicted the role of CD151 in extracellular matrix organization, hemidesmosome assembly, cellular component organization and cell junction assembly in breast cancer (Fig.1a). Over expression of CD151 was demonstrated using different techniques in high grade human breast cancers and triple negative breast cancer subtypes [13].

CD151 over expression enhances breast tumor formation, initiated by a range of oncogenes [6]. Elevated expression of CD151 associate with aggressive features [14], large tumor size, higher-nodal stage, advanced stage, absence of ER, PR and amplified HER-2 expression in invasive breast cancer [7]. However, in luminal and basal subtypes of breast cancer, CD151 associate with malignant phenotype. CD151 also contributes in driving tumor onset, survival and metastasis of ErbB2$^+$ breast cancer patients [15].

CD151 protein along with α3β1 influence tumor behavior of ductal carcinoma in situ [16]. ExosomalCD151 along with Tspan 8 is essential for the crosstalk between cancer initiation and microenvironment [17]. CD151 in association with integrins, uPAR, EGFR and MMPs aids migration, invasion and proliferation of tumor cells (Fig.1b) [18–20]. Van V Hua (2001) reported that lithium, carbamazepine and valproate decreased the expression of CD151 in rat brain [21]. It has been reported that glycosylation inhibitors also downregulated CD151 in U87MG and MDA-MB 231 cells [22]. CD151 gene silencing sensitized the breast cancer cells to small molecule inhibitors like camptothecin, FAK inhibitor (TAE 226), c-Met inhibitor(su11274) and EGFR inhibitors (laptinib and trastuzumab) [23].

## 2. Strategies for targeting CD151 in breast cancer

Studies have demonstrated that targeting CD151 by monoclonal antibody inhibited invasion [24], migration [25]

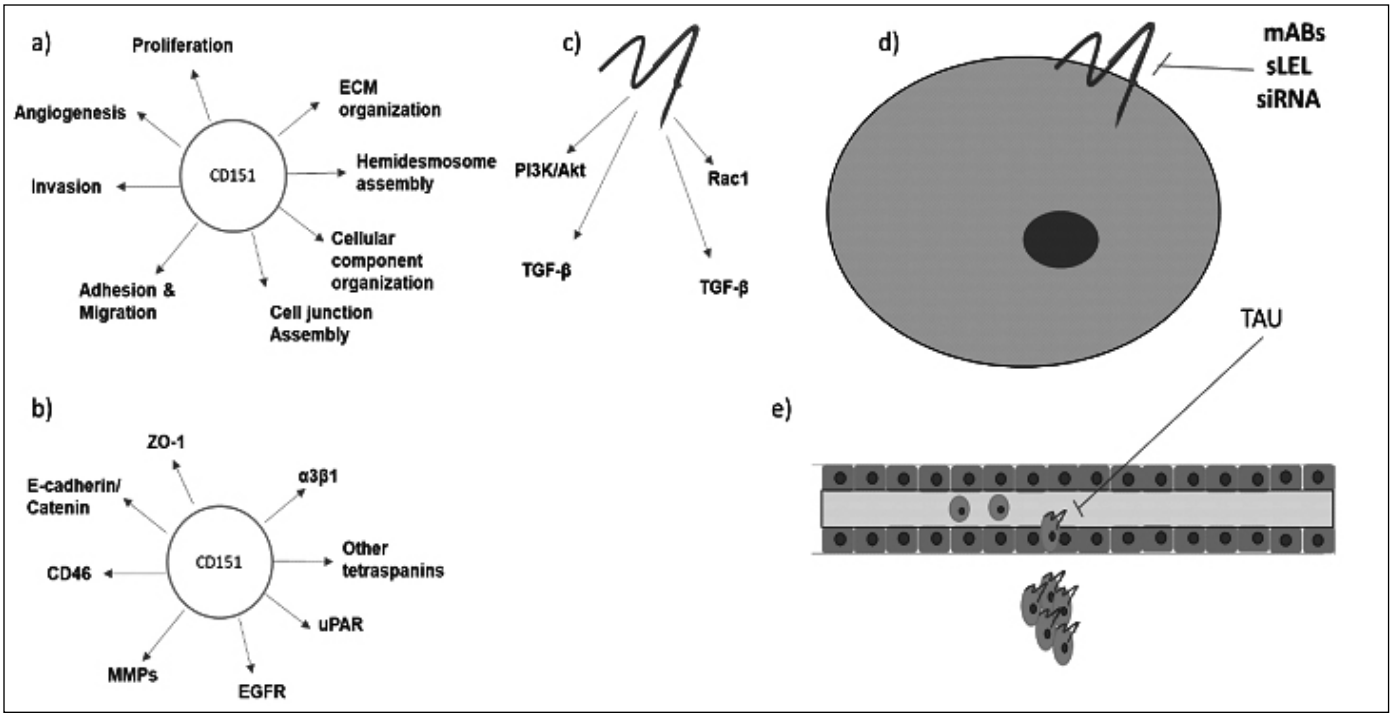


***Fig. 1:*** *Schematic model representing of the role of CD151-LEL and small molecule inhibitor, 2-thio-6-azauridine in targeting triple negative breast cancer. a) Role of CD151 in cellular functions. b) CD151 with its molecular partners in breast cancer. c) CD151 mediated signaling in cancers d) strategies for targeting CD151. e) Blocking of CD151-LEL with small molecule inhibitor, 2-thio-6-azauridine inhibits metastasis of triple negative breast cancer cells.*

and tumor progression [24] in various cancers. Removal of CD151 by antisense RNA inhibited phosphatidylinositol 3′-kinase (PI3K), Akt, and Rac1 mediated signaling pathways (Fig.1c) [13]. Small interfering RNA (siRNA) targeting CD151 inhibited proliferation, migration and induced apoptosis in cancer cells [26]. Ablation of CD151 diminished the metastasis in knockout mice [27]. Antibodies and siRNAs targeted at different regions of CD151 blocked variety of cellular functions in various cancers [28,29].

shRNA is highly effective tool used to silence the expression of specific genes. They can be designed with efficiency, specificity and without off-target effects. Recently, we constructed shRNA expression plasmid, which selectively silences CD151 gene expression [30]. We also constructed scrambled vector (pSV), in which CD151 target sequence was scrambled, to avoid off-target effects. The plasmids were authenticated by optimizing conditions for 100% transfection efficiency by close observation of cultured cells using microscope and also by Western blot and RT-PCR. CD151 shRNA sequence was analyzed using *In Silico* approach prior to construction for avoiding off-target sites. This shRNA at optimal conditions, significantly decreased the viability of MDA-MB-231 and MCF-7 breast cancer cells compared to pSV, in concordance with studies on human gastric carcinoma [31] but not affected either proliferation or survival of normal breast epithelial cell line, MCF-10A [2].

Silencing of CD151 using shRNA significantly reduced the adhesion of MDA-MB-231 cells to collagen-I and MCF-7 cells to fibronectin similar to other cancers [32]. Knockdown of CD151 also inhibited the invasion of both MDA-MB-231 and MCF-7 cells through matrigel in a Boyden chamber assay. This study observed that silencing of CD151 potentially reduced the migration of MDA-MB 231 and MCF-7 cells during wound healing in wound scratch assay. A recent study reported inhibitory effect of CD151 gene silencing on metastasis of MDA-MB-231 [33]. Another study, asserted that CD151 overexpression considerably enhanced the intercellular adhesiveness and delayed wound healing [34].

shRNA-mediated silencing of CD151 significantly inhibited the proliferation of MCF-7 cells by halting cell cycle at G2/M phase. Further, CD151 knockdown profoundly caused apoptosis in MCF-7 cells [35]. This study observed that expression of c-myc, a key survival protein was reduced with shRNA against CD151 in MCF-7 cells. This study also noticed a moderate decreased in the expression of CD151 partners, α3 and β1 integrins

with CD151shRNA, which may affect signaling pathway regulating cytoskeleton in breast cancer [36].

Clinical observation over decades suggested that angiogenesis is indispensable for tumor progression [37]. ER status has correlation with microvessel count in some breast tumors [38] but not in node negative breast carcinoma [39]. shRNA transfection markedly decreased the branching of new capillaries in MCF-7 cells with significant reduction of VEGF expression, a key factor of angiogenesis. The effect of VEGF expression on survival varies with intrinsic subtypes of breast cancer and the prognostic significance of VEGF expression in breast cancer remains controversial [40]. Some studies have stated that VEGF expression is negatively correlated with tumor progression in ER-positive breast cancers [41], but high-priority therapeutic target of some other breast cancers [42]. However, the current study showed possible role of CD151 in angiogenesis induced by MCF-7 cells using *in vitro* and *in vivo* similar to earlier studies [43].

## 3. Strategies for targeting LEL of CD151 in breast cancer

CD151 is enriched in distinct plasma membrane domains called tetraspanin web [44]. The schematic model of CD151 was extrapolated from the crystal structure of the tetraspanin CD81 [24]. It has a short cytoplasmic N- and C-termini and one small extracellular loop (SEL) and one large extracellular loop (LEL). Some important progress achieved recently concerning the LEL that bears an important part of interaction capability [45]. LEL protrude on one side of plasma membrane represent functional specialization for interaction with cholesterol, proteins and membrane microdomains [46]. Further, LEL with unique cysteine motif is required for interaction of CD151 with partner proteins and to promote several signaling pathways [47]. The LEL of CD151 contains functionally important sites [48], which makes LEL as a novel potential therapeutic target of metastatic cancers.

The tetraspanin web can be selectively disrupted by targeting LEL [47] or by silencing CD151 using siRNA [13,49]. Previously, LEL was targeted by silencing CD151 with siRNA/shRNA or by using monoclonal antibodies [24] and recombinant soluble LELs (Fig.1d) [50]. However, large size of mAbs decreases the penetration into malignant tissues and thus reducing the therapeutic efficiency [29]. The shRNA has some limitation in *in vivo* models especially delivery to specific tumor region [51]. Targeting CD151 with these agents can also disturb the normal functions of platelets in cancer patients [52]. Alternatively, strategies targeting at specific CD151-partner interaction could be worth exploring. Previously, small molecule inhibitor, benzyl salicylate was discovered by virtual screening as a lead of CD81-LEL structure, which has 30% similarity with CD151-LEL [53,54].

Recently, LEL portion of CD151 was expressed and purified from bacterial system [55]. Membrane modeling was used to predict the structure of EC2 over the whole superfamily [56]. Recently, the 3D structure of extra cellular loop of CD151 was modeled with satisfactory number of residues in favored (82%) and allowed regions (12%) using comparative modeling with active site residues: Tyr 34, Cys 174, Gly 193, Cys 156, Trp 169, and Asn 200 [57].

As small molecule inhibitors possess high penetration capacity, rapid inactivation of targets, and extensive biochemical effects at different stages of cell cycle and also targeting cancer cells at execution point [58]. Computer-aided drug screening against 3-D structure of CD151-LEL predicted small molecule inhibitor, 2-thio-6-azauridine (TAU) as potential lead. It presented a specific effect on viability of TNBC cell lines with minimal effect on normal breast epithelial cell line. Interestingly, it exhibited anti-proliferative activity comparable to paclitaxel with arrest of cell cycle at G1 phase and induction of apoptosis. TAU reduced the CD151 together with cell junction scaffold proteins, CD46, E-cadherin, β-catenin and ZO-1 in TNBC.

In summary, on the basis of available data and the comprehensive prerequisite for breast cancer cells, we proposed that CD151-LEL may contribute to metastasis of TNBC through interaction of specific binding partners. Further, 2-thio-6-azauridine mediated blocking of CD151-LEL may disturb the molecular interactions linking CD151 and its binding partners and subsequently inhibits triple negative breast cancer metastasis (Fig.1e). Finally, CD151 -LEL can be suggested as an elegant therapeutic target for small molecule-based therapy in TNBC. This study also showcases the CD151 as a potential target of triple negative breast cancers.

## 4. Future prospects

Treatment of TNBC at early stage can increase the survival rate, avoid metastasis and requires less extensive treatment. However, early detection is one of the major challenges in the struggle against this disease. These facts underscore the pressing need of novel strategies and technologies for treatment of TNBC at early stage.

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## Conflict of interest:

No conflict of interest is declared.

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# Protective effects of aqueous and ethanol extracts of Ficus religiosa Leaves and Bark on $H_2O_2$-induced oxidative DNA damage in human lymphocytes by comet assay

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**Abstract**

The Ficus religiosa leaves and bark were evaluated for their anti-genotoxicity properties by using various extractants. The ethanolic extract of the leaves and bark of the plant was found to exhibit the desired nature of the activity.

## 1. Introduction

Genetic Toxicology is the modern branch of toxicology, primary objective of genetic toxicology is to detect and analyze the hazard potential of those agents that are highly specific for interactions with nucleic acids and produce alterations in genetic elements at subtoxic concentration [1,2]. Among the known techniques in genetic toxicology, the come assay is one of them it is also called as single-cell gel electrophoresis. It was first developed in 1984 [3] and later modified 1988 [4]. It is a non-invasive technique compared with the other techniques (chromosomal aberrations, micronucleus) which require larger samples (2-3ml) as well as proliferating cell population (or cell culture). The assay has been widely used in studying DNA damage and repair in healthy individuals, in clinical studies as well as in dietary intervention studies and in monitoring the risk of DNA damage resulting from occupational exposures, environmental, oxidative damage or life style. Ficus religiosa is one of the most religious trees in Indian Hindu community and is almost available in every Hindu temple for worship. Besides of its mythological qualities, it is a well-known ethnomedicinal tree used in ayurveda. It is used in the preparation of traditional medicine for more than 50 types of disorders. The present work is designed to test the antigenotoxic nature of the Ficus religiosa leaves and bark.

## 2. Materials and Methods

Fresh leaves and bark of Ficus religiosa were collected from Draksharam, Bhimeswara swamy temple. The leaves and bark were washed under running tap water, air dried and powdered finely. The aqueous and ethanolic extracts of the Ficus religiosa leaves and bark were prepared by soxhlet apparatus. The fresh blood was taken from volunteers and the human peripheral blood (PBL) lymphocytes are extracted [5]. The Ficus extracts are first tested for genotoxicity and later for antigeno toxicity. The genotoxic nature is tested by treating the sample with the extracts at different concentrations (150µl, 200µl, 250µl, 300µl) using comet assay. The antigenotoxic is tested by treating the lymphocytes with H2O2 (25µl) after 30 min added leaf, bark aqueous and ethanolic extracts at different concentrations and it is cheeked with same comet assay (Singh et al.,1988).

## 3. Statistical analysis

The percentage of inhibition calculated by using the following formula:

Inhibition (%) = a – b/a – c × 100

Where, a = Tail length induced by $H_2O_2$ (positive control); b = Tail length of the compound in the presence of $H_2O_2$; c = Tail length of the negative control.

## 4. Results

Present study was undertaken to evaluate anti-mutagenic potential of *Ficus religiosa* Leaf Ethanol Extract, Leaf Aqueous Extract, Bark Ethanolic extract and Bark Aqueous Extract at different concentrations against $H_2O_2$ in human PBL by using Comet assay. First we find genotoxic concentrations of the $H_2O_2$. We treated Human PBL with 5µM to 30µM concentrations of $H_2O_2$ (Table 1) for 30 µM had proved its genotoxic nature. We tested these extracts can ameliorate the DNA damage induced by hydrogen peroxide in cultured human PBL by using comet assay. Lymphocytes without treatment observed tail length was 0.41± 0.12µm. Lymphocytes treated only with 30 µM concentration of hydrogen peroxide were used as the positive control (tail length 4.05 ± 0.94 µm). Lymphocytes treated with DMSO (comet tail length 0.61 ± 0.14 µm) were used as negative control.

To assay all extracts genotoxicity in PBL were evaluated using the comet assay. For mutagenic effect of extracts, a range of four concentrations from 150 to 300µg/

mL were tested (Table 1). As we observed, the 150, 200 µg/mL concentrations of the extract were nearly to that found in negative control (Table 2) and could be considered as non-genotoxic in both the assays.

To better express the antigenotoxic potential of the all extracts, the percentage inhibition in the number of measured aberrations was calculated. Among the four extracts of Ficus religiosa Leaf Ethanol extract at 300µg/ml concentration showing 86.2% inhibition against $H_2O_2$ induced human PBL damage. The nature of antigenotoxicity is increasing with the increasing extarct concentration. Even at the low concentration (150µg/ml) of this leaf ethnol extract, significant inhibition rate is observed (33.8%) (Table 3).

**Table 1: Effect of Hydrogen Peroxide on human PBL**

| Hydrogen peroxide | cells counted | Tail length ±SE |
|---|---|---|
| Untreated | 100 | 0.4 1± 0.12 |
| 5 µM | 100 | 0.85 ± 0.16 |
| 10 µM | 100 | 1.46 ± 0.33 |
| 15 µM | 100 | 2.56 ± 0.67 |
| 20 µM | 100 | 3.15 ±0.91 |
| 30 µM | 100 | 4.05 ± 0.94 |

**Table 2: Non-mutagenic effects of all the extracts by using comet assay**

| Compounds | Tail length ±SE |
|---|---|
| Untreated | 0.4 1± 0.12 |
| DMSO | 0.61 ± 0.14 |
| L.Et (150) (µg /ml) | 0.71+/-0.26 |
| L.Et (200) (µg /ml) | 0.82+/-0.29 |
| L.Et (250) (µg /ml) | 0.84+/-0.28 |
| L.Et (300) (µg /ml) | 0.98+/-0.34 |
| L.Aq (150)(µg /ml) | 0.71+/-0.27 |
| L.Aq (200) (µg/ml) | 1.05+/-0.31 |
| L.Aq (250) (µg/ml) | 1.25+/-0.29 |
| L.Aq(300) (µg/ml) | 1.35+/-0.39 |
| B.Et(150) (µg/ml) | 0.49+/-0.18 |
| B.Et (200) (µg/ml) | 0.61 ± 0.24 |
| B.Et(250) (µg/ml) | 0.75 +/-0.28 |
| B.Et (300) (µg/ml) | 0.91+/-0.34 |
| B.Aq (150) (µg/ml) | 0.88+/-0.28 |
| B.Aq (200) (µg/ml) | 0.99+/-0.33 |
| B.Aq (250) (µg/ml) | 1.10+/-0.38 |
| B.Aq (300) (µg/ml) | 1.14+/-0.41 |

**Table 3 : Antigenotoxic effect of following extracts against H2O2 Induced DNA damage**

| Hydrogen peroxide (20 µM) + Extract (µg /ml) | Tail length ±SE | % of inhibition |
|---|---|---|
| Untreated | 0.4 1± 0.12 | NS |
| L.Et 20+150 | 2.29+/-0.56 | 33.8 |
| 20+200 | 1.91+/-0.39 | 48.8 |
| 20+250 | 1.34+/-0.31 | 71.2 |
| 20+300 | 0.96+/-0.31 | 86.2 |
| L.Aq20+150 | 3.00 +/-0.59 | 27.7 |
| 20+200 | 2.95+/-0.54 | 39.3 |
| 20+250 | 2.09+/-0.39 | 41.7 |
| 20+300 | 1.94+/-0.35 | 47.6 |
| B.Et 20+150 | 1.99+/-0.38 | 45.6 |
| 20+200 | 1.45+/-0.24 | 66.9 |
| 20+250 | 1.04+/-0.16 | 83 |
| 20+300 | 0.98+/-0.19 | 85.4 |
| B.Aq 20+150 | 2.04+/-0.33 | 43.7 |
| 20+200 | 1.87+/-0.38 | 50.3 |
| 20+250 | 1.81+/-0.26 | 52.7 |
| 20+300 | 1.51+/-0.41 | 64.5 |

*L.Et = Leaf Ethanol Extract; L.Aq = Leaf Aqueous Extract; B.Et = Bark Ethanol Extract; B.Aq = Bark Aqueous Extrac: NS-Non significant; All the comets calicualted for 100 cells;*

## Conclusions

The leaf and Bark ethanol extracts showing high antigenotoxic property when compared to the leaf and Bark aqueous extracts. So the use of these extracts in the drug preparation of traditional medicine is more useful.

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**Dr. Sabitha.Y Ph.D.** *Founder and Managing Director of Ciencia Lifesciences. In 2015, started Ciencia life sciences mainly it works for research out sourcing, Academic traing and Projects for students in all Life sciences branches including Bioinformatics. My Career Started with GVK Bioinformatics division in 2002 and have experienced the whole methodologies of Bioinformatics. Awarded PhD from JNTU on the topic of Head neck cancers and smoking effect. As Research Associated in Indo American Cancer Hospital and Mahavir Hospital and Research centre have been worked. Over the my career, I have published 10peer-reviewed articles and participated in numerous conference presentations (Got the best poster in the ICOC 2007 and 3rd best poster in TWAS 2005) on the topic of Head and neck cancer and Bioinformatics. My research primarily falls into the Cancer, Bioinformatics, Development of Probiotics and Biofertilizers.*

# Intelligent Polymer Nanostructures to Treat Complex Diseases

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**Abstract**

The future of polymeric smart nano drug delivery systems can be regarded with optimism. The nanosystems that find various applications in medicine can be made more effective by adapting changes in the designing route to make them in to smart systems that are responsive to stimuli like size, concentration and pH.

## 1. Introduction

Nanotechnology, the area of research and technological developments at atomic, molecular, and macromolecular scales (1–100 nm), is being applied extensively to extending products lives, targeted and controlled drug administration to affected sites, improved performance, increase safety and patent adherence, reduce health care costs, in the fields of molecular biology and allied areas**.** The effectiveness of nanosystems can be improved by making them into smart systems i.e., designing stimuli responsive nanosystems which shows response to external or internal stimuli such as pH, temperate, concentration, enzyme etc. and allows controlled and targeted delivery of drug to desire site. Presently, a large variety of stimuli responsive polymers are used to develop smart nanosystems because their properties can be easily molded as well as they show drastic physical and chemical changes in macroscopic material properties response to physical stimuli (temperature, pH, light), chemical stimuli (various ''signaling'' molecules), or biological stimuli (enzymes) due to formation or breaking of secondary forces (hydrogen bonding, hydrophobic effects, electrostatic interactions, etc), changes in individual chain dimensions/size, secondary structure, solubility, or the degree of intermolecular association, changes in hydrophilic-to-hydrophobic balance, conformation, solubility, degradation, and bond cleavages, well as they show reversibility (the ability of the polymer to return to its initial state upon application of a counter-trigger). The forthcoming sections discuss some typical type of stimuli responsive drug delivery systems.

## 2. Magnetic field responsive drug delivery systems

The wide interdisciplinary world of nanoscience has experienced a strong development during several last years. One exciting topic is the possibility of using nanoscale magnetic materials for biomedical applications**.** The development of nanotechnology along with magnetism has opened new windows for sophisticated biomedical applications such as diagnostic, therapy etc. Nanoparticles offer a high potential for several biomedical applications such as cellular therapy, cell labeling, and targeting as a tool for cell-biology research, tissue repair, drug delivery, magnetic resonance imaging (MRI), hyperthermia, magnetofection; etc.

## 3. pH responsive drug delivery systems

Those polymers which contain pendant acidic (–COOH, $–SO_3H$) and swell in basic pH) or basic groups ($–NH_2$) and swell in acidic pH) i.e., polyelectrolytes show pH sensitivity because they can either accept or release protons in response to changes in environmental pH that vary their dimensions with the changes in the pH of the surrounding media. The external pH increases the release of drug if polymer nanoparticles contain weakly acidic (anionic) groups, but decreases if polymer nanoparticles contain weakly basic (cationic) groups as it affects the process of either gel swelling or deswelling. The pH sensitive hydrogels contain acidic or basic groups bound to the polymer chains. The acidic groups on the chains deprotonate at high pH, whereas the basic groups protonate at low pH**.** However, in basic solutions, the acidic groups bound to the polymer chains deprotonate, the $H^+$ ions come and combine with $OH^-$ to form $H_2O$. Charge is compensated by cations that enter the gel together with another $OH^-$ ion. The increased cation concentration gives rise to an osmotic pressure that causes the gel to swell/deswell. As the concentration of different ions changes inside the gel with time, the osmotic pressure changes and thus the deformation of the hydrogel which generates the elastic restoring force of the network also changes with time, can be read out as changes of the solution turbidity. Finally, an equilibrium is established when the elastic restoring force of the network balances the osmotic forces.

It has also been seen that gels swell faster in the presence of buffered solutions. pH-sensitive nanoparticles are very effective for delivery of drug to a specific site in the gastrointestinal tract, for cancer and tumor because extracellular pH of tumors (6.8–6.9) is more acidic than both tumor intracellular pH (7.2) and normal extracellular

tissues (7.4). Living system has different specific pH in different body part ranging from 1.2 to 7.4. Moreover, it is found that incorporation of a small amount of pH-sensitive ionizable groups, such as carboxyl and amino groups, into thermo-responsive polymers can offer a combination of different stimuli-responsive properties.

## 4. Temperature responsive drug delivery systems

Physiologically, Temperature is a very important stimuli trigger for smart drug delivery systems as it may act as both an external and internal stimulus. When Prostaglandin E2 concentration elevated in the brain then it mediates the body temperature by altering the firing rate of neurons that control thermo-regulation. Thermoresponsive hydrogels (undergo a coil-globule transition in aqueous solution at temperature values which is known as lower critical solution temperature (LCST)) are very versatile and important class of stimuli-responsive crosslinked, three dimensional polymer networks that reversibly swell with and then expel aqueous media in response to temperature changes which have been utilized in a wide variety of biomedical and drug delivery applications.

The common characteristic of temperature-sensitive polymers is the presence of hydrophobic groups, such as methyl, ethyl and propyl**,** ability to form secondary hydrogen bonding complexes that exhibit a positive sigmoidal swelling transition with temperature**,** and their rapid swelling transition with temperature, termed the 'zipper effect', which is due to the long range hydrogen bonding order makes these polymers an ideal system for on/off controlled release applications. At lower temperatures, water molecules in the vicinity of hydrophobic polymer chains are highly hydrogen bonded. Since these hydrogen bonding interactions lower the free energy of mixing considerably, the polymer chains dissolve or swell in water at low temperatures. At higher temperatures, the hydrogen bonds weaken; at the same time, the tendency of the system to minimize the contact between water and hydrophobic surfaces, i.e., the hydrophobic interaction increases. As a result, on heating a polymer solution or a polymer gel, a transition from swollen to collapsed state occurs at a critical temperature.

Poly (N isopropylacrylamide) PNIPAam referred as a smart material exhibits LCST at around 31 ◦C and is the most thermoresponsive polymer which is used in many biomedical applications. It undergoes response to change in temperature because its amide groups release the water molecules when temperature is increased above LCST and polymer-polymer interactions become dominant make it into shrink form. While some other polymers show behavior opposite to LCST, they possess an upper critical solution temperature (UCST), below this temperature they collapsed.

## 5. Light/photo responsive drug delivery systems

Those polymers which contain azobenzene, spirobenzopyran, triphenylmethane or cinnamonyl, titanium dioxide ($TiO_2$) and quantum dots are coined as light/photo-responsive polymers because they show change in their properties such as size and shape, or forming ionic or zwitterionic species, when irradiated with light of the appropriate wavelength. Recently, light sensitive nanoparticles have gain much importance for use in biomedical applications (reversible optical storage, polymer viscosity control, photomechanical transduction and actuation, bioactivity switching of proteins, tissue engineering, and drug delivery) because it is a very easy stimuli to controlled the drug delivery as its intensity and wavelength can easily be controlled through the use of filters. Several light-sensitive particles have been encapsulated within vesicles that cause a physical change upon light exposure, leading to vesicle disruption.

## 6. Electric field responsive drug delivery systems

Electro-responsive polymers have been investigated as a form of smart nanomaterials for bio-related applications such as drug delivery systems, artificial muscle, or biomimetic actuators because they can swell, shrink, or bend in response to an electric field. These polymers based nanoparticles are able to transform electrical energy into mechanical energy so they are widely using in biomechanics, artificial muscle actuation, sensing, energy transduction, sound dampening, chemical separations, and controlled drug delivery.

## 7. Conclusions

In this study a number of examples of stimuli responsive drug delivery systems have been discussed but the future of polymeric smart nano drug delivery systems can be regarded with optimism, not least because of the simplicity of introducing structural changes develop inherent advantages. However, the design and development of stimuli responsive nanodrug delivery systems should follow specific standards i.e., they should be able to elegantly address the challenging issues of current nanoparticulate drug formulations including aspects of preparation and drug loading, in vivo stability, tumor-targetability, tumor cell uptake, and intracellular drug release.

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# Radiation processed polymeric materials for healthcare applications

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**Abstract**

Radiation processed polymeric biomaterials in form of implants, controlled drug delivery devices, grafted matrices, contact lenses, antibacterial fabrics, hydrogels, sensors etc. find wide applications for healthcare. Electron beam irradiation and gamma ray irradiation both have been extensively used for synthesis and modification of biomaterials for healthcare. As radiation induced chemical reactions do not involve any chemical initiators/sensitizers the products obtained through radiation induced grafting or crosslinking are of high purity. The radiation processing does not require special sterile production rooms but still enables to obtain sterile products with high through-puts. The sterilization process provides better process control as only one parameter the time of irradiation is to be monitored. New biomaterials with bulk or surface modification can be synthesized with possible additional advantage of concurrent sterilization using high energy radiation. Radiation processed polymeric biomaterials hold bright future prospect in applications like tissue engineering, early diagnostic and pharmaceutical bioprocesses. The article presents an overview of different activities presently being pursued in Radiation Technology Development Division, BARC related to radiation processed materials for healthcare applications.

## 1. Introduction

Radiation processing of polymeric materials, blends and composites for healthcare has several distinct advantages over conventional thermo-chemical processes. Radiation processing being an additive free process leads to high purity products. It is room temperature process thus thermo-sensitive systems can be processed. Also radiation processing allows radiation sterilization of products during processing itself [1]. Polymers undergo radiation induced degradation, crosslinking or can be grafted under suitable conditions depending on irradiation conditions and polymer properties.

Such radiation induced transformations in polymers and polymer composites can be utilized to develop advanced sensors for the detection of metabolic biomarkers. Recently, a radiation crosslinked fluoroelastomer conducting composite was developed to detect 2-propanone vapours over a wide concentration range. Such sensors find use for several healthcare applications, particularly in the early detection of acute conditions of diabetes. For example, diabetic ketoacidosis is a condition where enhanced sugar levels in blood, results in elevated acetone expulsion in exhaled breath due to lack of insulin [2-3]. Non-invasive diagnosis of disease such as diabetes through exhale breath is desired for early detection and patient comfort [4-7]. These sensors are chemiresistive and can be integrated in an array. Notably, low cost, short response time, easy production, real time monitoring and possibility of easy integration into a chemiresistive array are main advantages of radiation processed chemiresistive sensors over others [8-10].

Hydrogels are three dimensionally crosslinked polymers that absorb significant amount of water while retaining their 3-D network structure. Typically they are two component systems where one of the components is a hydrophilic polymer, rendered insoluble in water due to three dimensional network structure interconnecting its polymeric chains, while the other component is water. These systems may swell and absorb significant amounts of water, saline solutions or physiological fluids while retaining their shape [11]. The interactions that lead to water absorption include process of hydration that depends on presence of chemical groups such as $-OH$, $-COOH$, $-CONH_2$ and $-SO_3H$ on the polymer. On the other hand forces that inhibit hydrogel swelling depend on the extent of covalent bonding between polymer chains as well as hydrophobic interactions between polymer chains. The swollen state results from a fine balance between the dispersing forces acting on hydrophilic groups and the cohesive forces preventing dissolution of polymers in water. The desired characteristics of hydrogels depend on final use of the matrix. However, generally, they should have optimum swelling degree, good water retention, desired swelling kinetics, good mechanical strength and finally should be biocompatible [12].

Radiation grafting of polymers envisages covalently linking a new polymer to existing backbone polymer using high energy radiation as a tool with an aim to

produce a product with additive properties of both the constituents [13]. Cotton fabrics are frequently used as clothing material because of their natural abundance and inherent properties like breathability, moisture absorbency and comfort [14]. However, cotton is amenable to bacterial and fungal attack under moist conditions, which leads to mildew, causes bad smell and decreases cloth life. To avoid bacterial and fungal attack antibacterial property can be introduced into the fiber, either at the manufacturing step itself by entrapment of antibacterial chemicals, by coating the finished product with antibacterial compounds or by grafting antibacterial quaternary ammonium salts (QAS) [15]. The grafted antibacterial cotton finds application, such as clothing for hospital staff and patients, hospital beddings, ladies tights, shoe linings, sleeping bags, sports clothing, armbands etc.

Availability of high energy sources like gamma radiation plants & electron beam (EB) machines on one hand and cheap plastic medical devices (syringes, sutures, gloves, gauze, cotton) on other hand has ushered the era of single use medical devices. This in turn has caused significant improvement in prevention of several diseases. The high energy radiation due to its deep penetration power is able to cause single/double strand damage or damage to the bases of pathogenic species rendering them incapable of proliferating thus causing effective prevention of diseases [16].

## 2. Radiation processed chemiresistive sensors for 2-propanone sensing

Fluorocarbon elastomer (FCE)/nanocarbon black (NCB) composites were synthesized via melt compounding and crosslinked by high energy gamma irradiation to different extents. Conducting composites in different parts of the percolation profile were chosen to understand the effect of crosslinking extent on sensing response of the composite. It was found that the radiation dose markedly affects the sensing response of these sensors. As shown in figure 1 the sensor showed highly selective response for 2-propanone while no response was noted against benzene, toluene, ethyl benzene and xylene (BTEX) under static VOC conditions. It was found that the sensing response had a strong correlation with the FCE-analyte interaction parameter ($\chi_{12}$). Extensive mass uptake, impedance spectroscopy and morphological analysis were employed to understand the contact resistance, polymer chain relaxation, analyte diffusion and dispersion. The dynamic mechanical response of the system indicated that an absorbed dose of 300 kGy dose severally impairs the segmental motion (figure 2) [17].

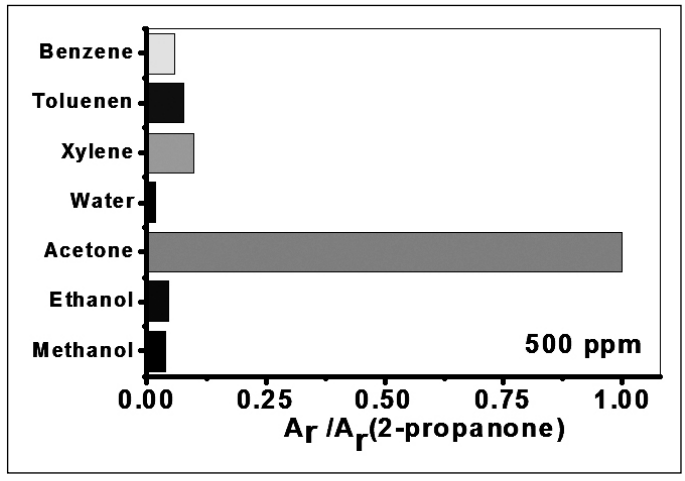


***Fig. 1:*** *Variation in relative change in resistance for chemiresistors {(FCE: NCB::65:35); Dose 100 kGy} against different VOCs. [VOC]=500 ppm, Static setup (Reprinted from Mondal et al.; Sensors and Actuators B 265 (2018) 193-203; with permission from Elsevier)*

## 3. Hydrogels for controlled drug delivery systems (CDDS)

Conventional methods of drug administration such as oral, subcutaneous, intramuscular or intravenous injections have several drawbacks, such as necessity of multiple dosing, fluctuating drug concentration in plasma and tissue and drug distribution to non-target tissues. In recent years, much attention has been given to the investigation of novel methods of drug administration instead of conventional ones, in order to prevent ineffective administration and undesirable side effects. Immobilization of active moieties like drug, enzyme, proteins in suitable polymer matrices have been successfully attempted to meet the following two requirements (i) to prolong the duration of pharmacological efficacy of the moiety and (ii) to target the active moiety so as to deliver the drug efficiently and effectively to the desired organ or tissue. This can be achieved either by physical entrapment or through chemical bonding of the drug to polymer matrix.

In physical entrapment method the active moiety is physically trapped in a crosslinked network of hydrophilic polymer by irradiation of a mixture of monomer and active component. The diffusion of the active component depends on polymer matrix and the environmental conditions. The release of the drug can be controlled over a wide range by change of design and change of various factors in the polymer matrix such as molecular weight, copolymer composition, chemical structure, stereoisomerism, hydrophilicity, bio-degradability, porosity and the radiation dose. When the active component is radiation sensitive, the polymerization and crosslinking can be carried out in super cooled state (-80°C) in order to minimize radiation damage

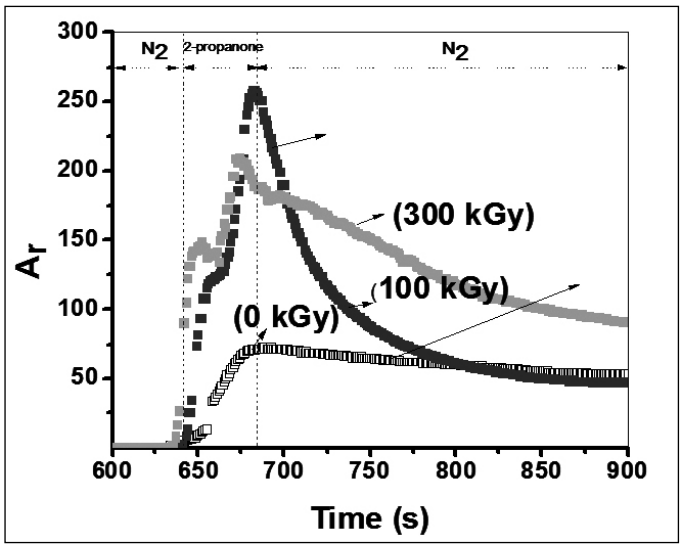


***Fig. 2:*** *Variation in resistance for (FCE: NCB::65:35) chemiresistors in presence of 2-propanone vapours for chemiresistors irradiated to different absorbed doses. The figure also shows the photographs of unirradiated disintegrated chemiresistor and retention of the integrity for irradiated chemiresistor after putting in 2-propanone for 30 min. (Reprinted from Mondal et al.; Sensors and Actuators B 265 (2018) 193-203; with permission from Elsevier)*

to the drug, or the drug can be subsequently incorporated in the matrix by swelling the polymer in a solution of the active component in an appropriate solvent. Radiation polymerization/crosslinking in super cooled state has been explored to design a porous matrix which due to release of trapped moiety by pore and partition mechanism overcomes the intricacies involved in geometrical design of CDDS. Figure 3 shows a protocol for synthesizing such porous beads at -78°C [18].

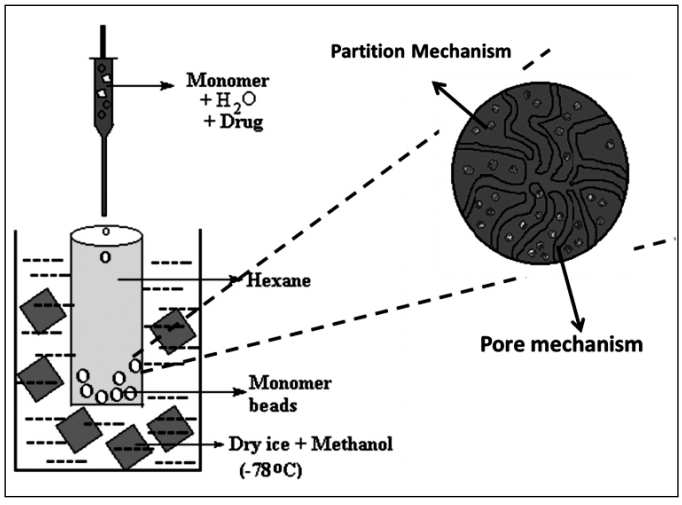


*Fig.3: Radiation induced synthesis of porous polymeric matrices of (2-HEMA+EGDMA+NVP/MMA) (Reprinted from Bhardwaj et al.; Pharma Times Vol. 48 (2016) No. 08 - August 2016; with permission from Elsevier)*

## 4. Wound dressings

Healing of wide and serious burn wounds is a difficult medical problem. The dressing used for protection of these wounds has to be changed regularly till healing takes place. BARC initiated the program of developing a synthetic polymer; poly vinyl alcohol (PVA) based additive free hydrogel for use as wound and burn dressings. The extensive research done on the radiation induced synthesis of this hydrogel resulted in development of PVA based hydrogel dressing that possesses excellent characteristics for the desired use. The product has been extensively tested in the local hospitals, more than 10,000 pieces have been supplied to various users [**19**]. The technology of production has been transferred to a private entrepreneur for production on commercial basis and the product is now available in the Indian market.

Biological and clinical tests have shown that these hydrogels have excellent bio-compatibility. The technology of producing HDR dressings using radiation offers some distinct advantages over conventional chemical processing such as

(i) The technology is simple, easy and clean.

(ii) There is no need to maintain special sterile rooms but the end product is sterile.

(iii) The production can be carried out as a continuous as well as a batch process.

## 5. Radiation grafted antibacterial textiles

Textiles with antibacterial or antiviral activity have become extremely important in the health protection of human beings. The antibacterial activity of textiles can be obtained through the antibacterial finishing of textile using antibacterial, agents or by incorporating them into synthetic fibers during preparation [20]. The main antibacterial agents include metals or metal salts, quaternary ammonium compounds (QACs), polybiguanides, N-halamine, chitosan and triclosan. The covalently linking antibacterial agents can provide permanent antibacterial finishing.

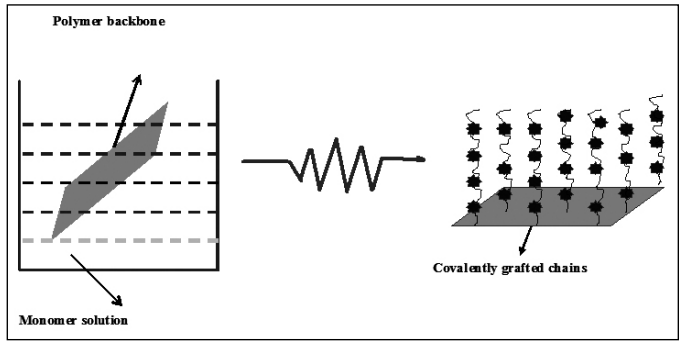


***Fig. 4:*** *Mutual radiation grafting process*

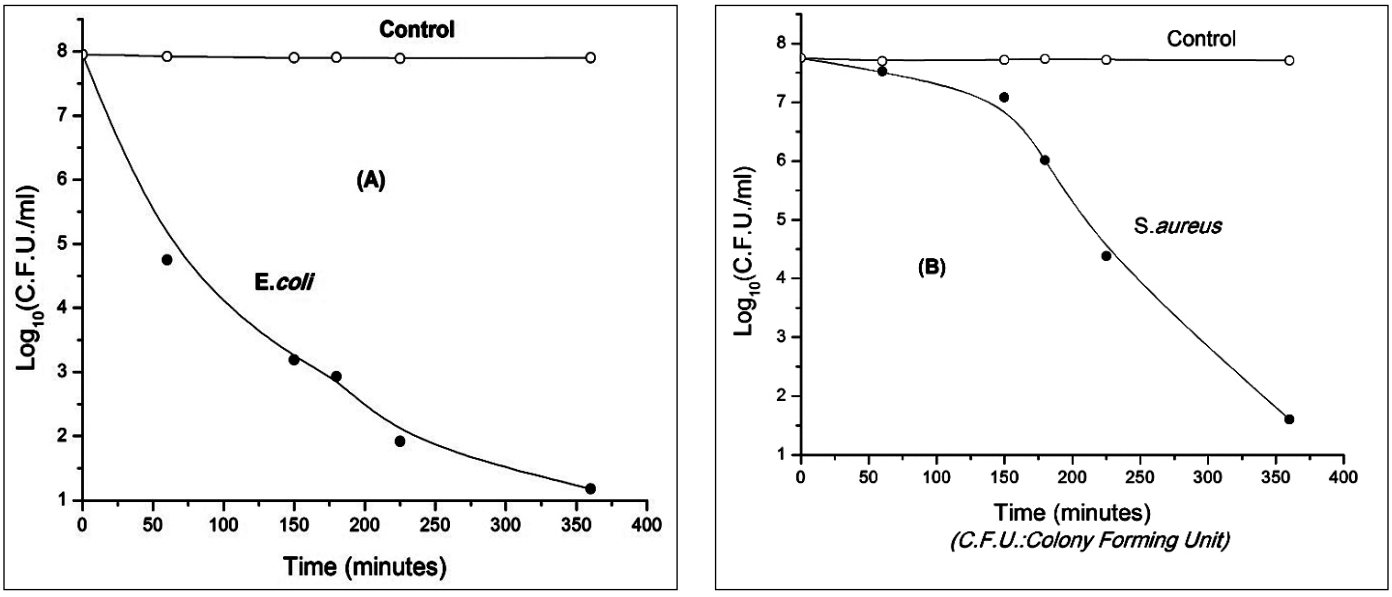


*Figure 5: Anti-bacterial activity of VBT-g-cotton (25% grafted) against (A) E. coli. (B) S. aureus (Reprinted from Virendra et al.; Radiation Physics and Chemistry 73 (2005) 175-182; with permission from Elsevier)*

**Table 1: Effect of VBT grafting extent on E. Coli cells (*Reprinted from Virendra et al.; Radiation Physics and Chemistry 73 (2005) 175-182; with permission from Elsevier)***

| Time of exposure (in hrs) | % Grafting | | |
|---|---|---|---|
| | 5 % | 13 % | 20 % |
| | Counts (CFU/ml) | | |
| 0 | $1.0x10^9$ | $1.1x10^9$ | $1.0x10^9$ |
| 2 | $2.8x10^8$ | $4.0x10^4$ | $1.0x10^5$ |
| 4 | $2.0x10^8$ | $1.2x10^4$ | $4.0x10^4$ |
| 6 | $7.5x10^8$ | --- | $1.0x10^4$ |

**Table 2: Effect of VBT grafting extent on S. aureus cells (Reprinted from Virendra et al.; Radiation Physics and Chemistry 73 (2005) 175-182; with permission from Elsevier)**

| Time of exposure (in hrs) | % Grafting | | |
|---|---|---|---|
| | 5 % | 13 % | 20 % |
| | Counts (CFU/ml) | | |
| 0 | $1.0x10^9$ | $1.0x10^9$ | $1.1x10^9$ |
| 2 | $1.0x10^9$ | $6.1x10^8$ | $1.0x10^9$ |
| 4 | $1.0x10^9$ | $1.8x10^4$ | $1.7x10^4$ |
| 6 | $8.0x10^6$ | $3.0x10^4$ | $1.6x10^4$ |

Grafting essentially involves attaching a polymer/ group of interest to the existing polymer backbone for the desired application. Intention here is to get a product which has properties of both the input materials economically. Figure 4 gives an established procedure for carrying out mutual radiation grafting of polymers.

Trialkyl ammonium chlorides have been reported to possess germicidal effect in dilute aqueous solutions. Thus radiation induced grafting of monomers like (ar-vinylbenzyl)trimethylammonium chloride (VBT), (2-(Methacryloyloxy)ethyl]trimethylammonium chloride} (MAETC), [2-(Acryloyloxyethyl)]trimethylammonium chloride (*AETC*) onto finished cotton cloth have been reported through mutual radiation grafting method to impart antibacterial property to cotton [21-23]. The grafting extent was found to be a function of irradiation parameters like dose, dose rate, irradiation ambiance and chemical parameters like monomer type & concentration, solvent, and homopolymerisation inhibitor type and concentration. The grafted matrices were found to bactericidal rather than inhibitory. The bactericidal activity against gram positive and gram positive bacteria for grafted matrix was different (figure 5) [**23**] and also time dependent (table 1 & 2) [23]. The bactericidal property was also a function of grafting extent (figure 6) [**22**] and the monomer grafted. The grafted cotton was observed to retain its antibacterial property even after several cycles of washing (figure 7) [23].

## 6. Radiation sterilization of medical products

Radiation sterilization of medical products is well established industrial process. ISOMED the first radiation plant for sterilization was setup in India by the department of Atomic Energy at Trombay with assistance of United Nations Development Programme in year 1974. As on today to cater to the large volume of sterilized single use

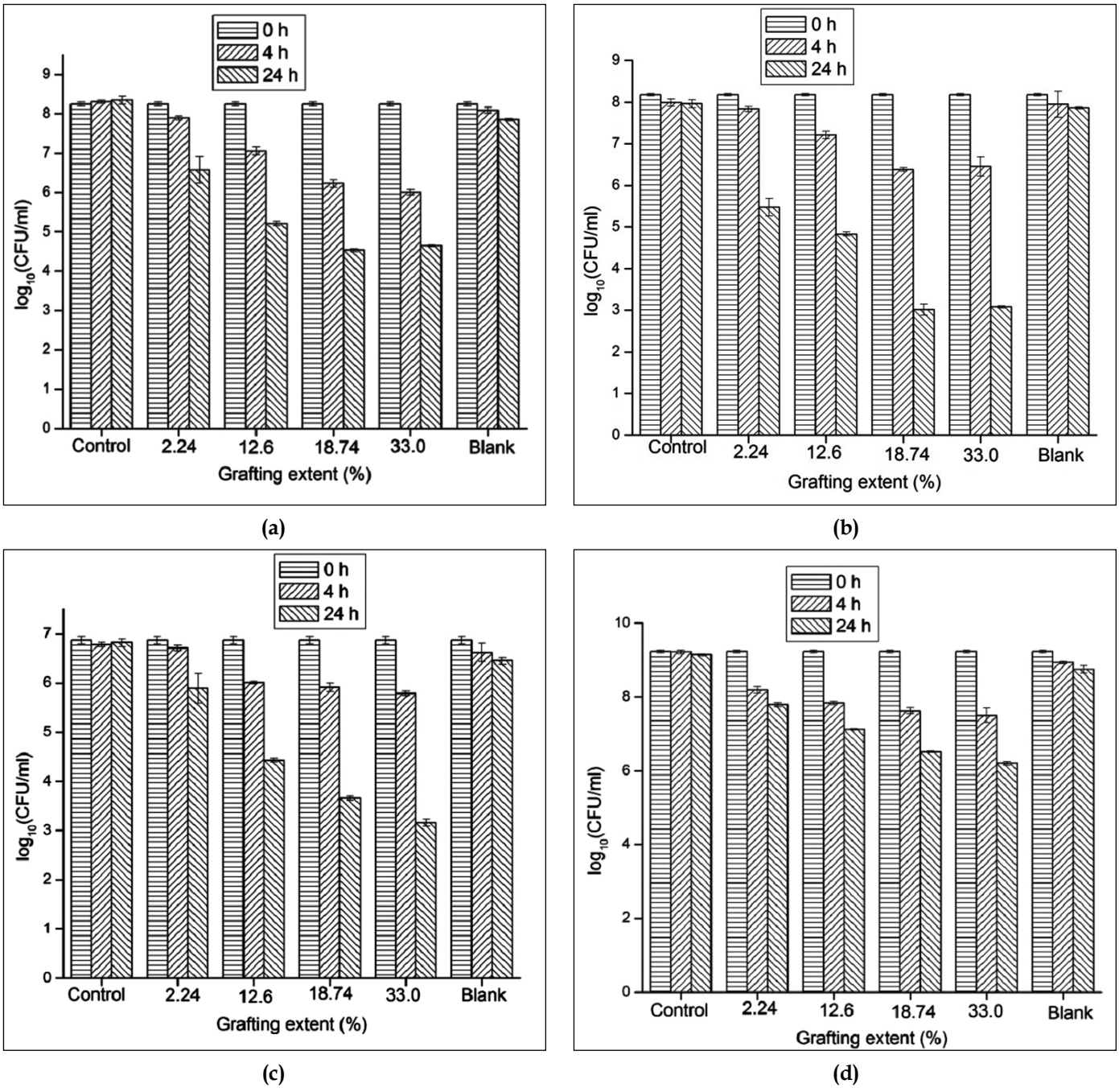


*Fig. 6: Anti-bacterial activity of MAETC-g-cotton against (a) E.coli (b) S. aureus (c) B. cereus (d) P. fluorescens (Reprinted from Goel et al.; Radiation Physics and Chemistry 78 (2009) 399-406; with permission from Elsevier)*

medical products 12 gamma sterilization plants [**24**] and six multi-purpose electron beam machines are in operation [**16**]. In an effort to further demonstrate the effectiveness of radiation sterilized product, particularly for the rural segment where infrastructural facilities are inadequate, a number of specific kits have been developed. One such product is gamma sterilized "Dai Kits" which contains radiation sterilized materials such as absorbent gauze, cotton, blades, umbilical tapes, soaps and drape sheets etc. normally required for delivery procedure in rural homes. The use of these kits is reported to have significantly reduced the infant morbidity and mortality rates in rural areas.

## 7. Outlook and future trends

Radiation and isotopes based techniques are well established for diagnosis and cure in healthcare sector. Better understanding of radiation effects on materials has provided further impetus to utilization of radiation processed materials for prevention, cure and diagnosis.

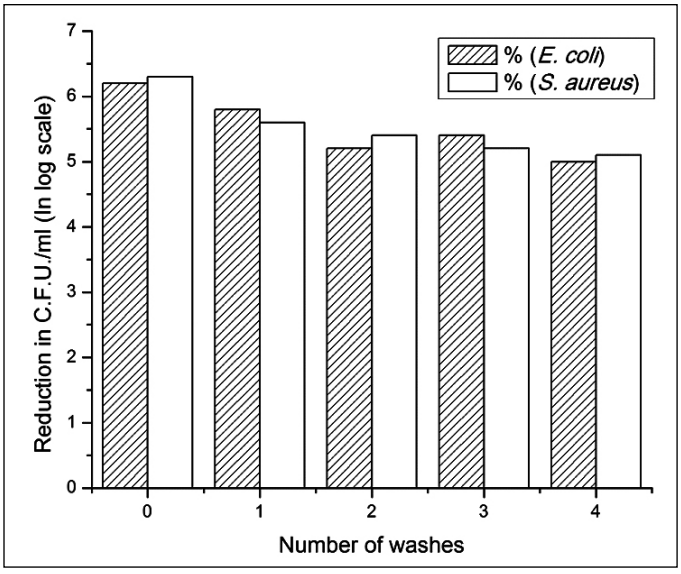


***Fig. 7:*** *Effect of washing on anti-bacterial activity of the 25% VBT grafted cotton(Reprinted from Virendra et al.; Radiation Physics and Chemistry 73 (2005) 175-182; with permission from Elsevier)*

Radiation sterilization of medical products has been success story and is today a billion dollar industry. Radiation processed products like hydrogels are making their presence felt in healthcare industry. The present focus is on radiation processing of natural polymers such as chitin/chitosan, starch and other water soluble polymers for synthesizing biocompatible devices. Synthesis of radiation processed medical grade highly radio-opaque but flexible composites for non-invasive detection of implants has been of interest for several groups worldwide. Radiation processed conducting composites capable of detecting volatile organic compounds in exhaled breathe which are specific disease markers have been proposed as an array for early detection of diseases in patient-friendly manner. Preparation of micro heterogeneous or micro porous polymer structures using sub-room temperature radiation processing technology is expected to be utilized for immobilization of living cells and bio-functional molecules in near future. The radiation processed high purity nano-matrices are among the suitable systems for targeted delivery. Besides radiation polymerization, crosslinking and grafting, radiation surface etching lithography, ion beam irradiation and laser irradiation would emerge as new frontiers for radiation processing techniques for healthcare applications.

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# Synthesis and characterization of Glucosamine stabilized Palladium ($Pd^0$) nanoparticles as efficient catalyst for reduction of nitro arenes

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**Abstract**

A facile synthesis of palladium nanoparticles and their catalytic application in the reduction of nitro arenes by D-glucosamine solution is reported. The size and shape tuned Pd nanoparticles were characterized by X-Ray Diffraction studies, Transmission Electron Microscopy and the size of the Pd Np were reported as 6 nm. Further, the catalytic performance of the palladium nanoparticles was optimized at room temperature for the hydrogenation of nitro arenes. The reusability nature of the catalyst was showcased that it retains its nature until the first three cycles.

**Key words:** carbohydrate, D-Glucosamine, palladium, nanoparticles, reduction, nitro compounds

## 1. Introduction

The remarkable applications of metal nanoparticles in areas of nanoscience and technology fascinated the researchers to focus on their synthesis[1–5]. Several physical and chemical methods are developed for the synthesis of metal nanoparticles with desired size and shape. Out of all, chemical reduction method is one of the best methods to produce metal nanoparticles in a large scale in which reducing agents like sodium borohydride, hydrazine, citrate, ascorbic acid[6–8] are used. Also, stabilizing agents like polymers and surfactants are employed to protect metal nanoparticles and prevent them from the aggregations[9]. But, still there are several environmental problems associated with these synthetic methods.

Recent reports have shown that transition metal nanoparticles are efficient catalysts for chemical transformations. Out of all, Palladium nanoparticles (PdNP) become favorable catalyst due to their relatively low price and high catalytic activity. Palladium nanoparticles are widely used as a catalyst especially for petroleum/ petrochemical and automobile catalytic converter[10] and in various organic reactions such as petroleum cracking process, Suzuki's coupling reaction, and hydrogenation[11–13]. Hence, researchers focused on production of very small size palladium nanoparticles in order to enhance catalytic activity via their high surface area[14]. Despite the availability of sizable number of reports on the synthesis of palladium nanoparticles, there is ascope to develop an efficient synthetic method with environmental friendliness to improve their catalytic activity.

The nitro compounds are the versatile intermediates found widespread in industrial application[15–17]. Amines and their intermediates are used as intermediates in the azo dye and pigment synthesis and also used in bioactive compounds, pharmaceuticals, agrochemicals and optical brighteners, as intermediate for photographic chemicals[18] [18–23]. Hence the reduction of nitro compounds to amines plays a vital role in chemical, petrochemical, pharmaceutical and food industries[19–21]. Normally the reduction of nitrobenzene is carried out under homogeneous conditions[22] and these homogeneous catalysts suffer from several disadvantages.

To overcome these disadvantages, we report an eco-friendly approach in the synthesis of Pd nanoparticles by D-glucosamine (a carbohydrate) to prepare well-stabilised nanoparticles. Also, the activity of Pd nanoparticle catalysts for the catalytic hydrogenation of nitro-benzene under mild conditions was studied. Moreover, the purpose of the work is to maximize the catalytic performance of the catalyst and to reuse, recycle the catalyst without or with a minimal degradation under mild operating conditions.

## 2. Experimental

### 2.1 Preparation of D-Glucosamine stabilized Pd nanoparticles

In typical synthesis process, 3.33 g D-Glucosamine was dissolved in 50 ml of distilled water taken in a round bottom flask under vigorous stirring. To this, 1.12 g of Pd (II) acetate [$(CH_3COO)_2Pd$, Aldrich with 99.9% purity] was added under vigorous stirring. The pH of reaction mixture was maintained at 10 was by using 25% $NH_3$ solution. The reaction mixture was then stirred for an additional

30 minutes at room temperature when the colour of reaction mixture slowly turns to dark gray, indicating the formation of Pd nanoparticles. Then the reaction mixture was transferred into a sealed tube and reaction was further preceded for 4 h at 180 ˚C. The reaction mixture was allowed to cool at room temperature. The resulting mixture was centrifuged and washed with distilled water and finally dried at 60 ˚C for 12 h. As prepared D-Glucosamine stabilized Pd nanoparticles were characterized by X-ray diffraction patterns (XRD), UV-Visible spectroscopy, transmission electron microscopy (TEM) .

### 2.2 Hydrogenation of nitrobenzene & substituted nitrobenzenes by D-Glucosamine stabilized by $Pd^0$ nanoparticles

The reduction reaction was carried out using Pd/ carbohydrate (D-Glucosamine), catalyst (50 mg) in 1mmol of nitro benzene and 20 ml of ethanol at room temperature in hydrogen atmosphere for 60 min under stirring condition. The completion of the reaction was monitored by TLC and after completion; catalyst was separated by centrifugation and subsequently washed with dichloromethane. The reaction mixture was diluted with water and product was extracted with dichloromethane (2 × 10 mL). The organic over layer was dried anhydrous sodium sulphate and evaporated under reduced pressure to afford the crude product. The crude product was purified by column chromatography and characterized by advanced spectroscopic techniques.

### 2.3 Characterizations

The UV-Vis spectra were recorded on Hitachi U-3010 spectrometer and FTIR spectra were recorded on Perkin Elmer Model impact 410 spectrometer. NMR spectra were taken from JEOL JNM FTNMR-400 MHz spectrometer. X-ray diffraction patterns (XRD) were carried out using a MINI FLEX RIGAKU MODEL X-ray diffractometer, at 2 degree per minute with Cu-Kα radiation (1.5418 A°) with scan ranging from $10^o$ to $80^o$.The surface morphology and particle size of D-Glucosamine stabilized Pd nanoparticles was investigated by transmission electron microscopy (TEM). Transmission electron microscopy images were obtained [TEM model FEI TECNAI G2 S-Twin] at an accelerating voltages of 120 and 200 kV.

### 2.4 Spectral data of synthesized compounds

Aniline (2a): IR (KBr pellet) $cm^{-1}$: 3430.61, 3036.26, 1601.72, 1274.47, 692.52 1H NMR (400 MHz/ $CDCl_3$) δ ppm): 7.01 (2H, t, J=8.62Hz) 6.50 (1H, t, J=8.20Hz), 6.41 (2H, d, J=7.64Hz), 3.30 (2H, br., s).13C NMR (100 MHz/ $CDCl_3$) δ ppm): 145.8, 128.2, 117.8, 115.5 Mass-ESI: 94.06 (M+H).

4-aminobenzaldehyde (2b): IR (KBr pellet) $cm^{-1}$ : 3425.50, 2950.40, 1710.58 ,1550.00, 1100.50, 820.65; 1HNMR (400 MHz/ CDCl3) δ ppm): 9.50 (1H, s), 7.50 (2H, d, J=8.42Hz) 6.40 (2H, d, J=7.88Hz)), 3.50 (2H, br., s); 13C NMR (100 MHz/CDCl3) δ ppm): 190.0, 150.0, 129.5 , 125.0, 115.5;Mass-ESI: 122 (M+H).

4-aminobenzonitrile (2c): IR (KBr pellet) $cm^{-1}$: 3477.81, 2213.96, 1514.17, 1212.42, 829.88; 1H NMR (400 MHz/ DMSO) δ ppm): 7.22 (2H, t, J=7.42 Hz), 6.60 (2H, t, J=6.84 Hz ), 3.88 (2H, br., s); 13C NMR (100 MHz/CDCl3) δ ppm) : 150.2, 133.0, 120.1, 114.5, 99.0; Mass-ESI: 119.06 (M+H).

4-chlorobenzenamine (2d):IR (KBr pellet) $cm^{-1}$: 3457.46, 2958.40, 1540.50, 1150.45, 820.48, 748.27; 1HNMR (400 MHz/ CDCl3) δ ppm): 7.50 (2H, d, J=8.20 Hz ), 6.00 (2H, d, J=7.86 Hz), 3.80 (2H, br., s); 13C NMR (100 MHz/CDCl3) δ ppm) :145.0, 128.5, 123.0, 115.5; Mass-ESI: 127 (M).

4-methoxybenzenamine (2e):IR (KBr pellet) $cm^{-1}$:(Fig 5.14) 3423.05, 2964.53, 2839.53, 1506.26, 1234.24, 1129.64, 826.06;1H NMR(400 MHz/ CDCl3) δ ppm):(Fig 5.15) 6.70 (2H, d, J=8.20Hz), 6.63 (2H, d, J=7.64Hz ), 3.75 (2H, br., s), 3.31 (3H, s);13C NMR (100 MHz/CDCl3) δ ppm): (Fig 5.16) 152.7, 140.0, 116.2, 114.5, 55.8; Mass-ESI: (Fig 5.17) 124.07 (M+H).

M -toluidine (2f): IR (KBr pellet) $cm^{-1}$ : 3465.45, 2986.50, 1578.95, 1140.43, 705.65; 1H NMR (400 MHz/ CDCl3) δ ppm): 6.70 (1H, dd, J=8.20 Hz), 6.30 (1H, dd, J=7.80Hz), 6.10 (1H, s), 6.15 (1H, d, J=6.42Hz), 3.80 (2H, br., s), 2.10 (3H, s);13C NMR (100 MHz/CDCl3) δ ppm) : 145.0, 135.5, 128.0, 120.0, 112.5, 23.5; Mass-ESI: 108 (M+H).

O-toluidine (2g): IR (KBr pellet) $cm^{-1}$: 3420.45, 2930.54, 2860.28, 1542.70, 1200.50, 1168.32, 750.30;1H NMR(400 MHz/ CDCl3) δ ppm): 6.70 (1H, dd, J=8.20 Hz), 6.60 (1H, d, J=7.42Hz ), 6.45 (1H, dd, J=6.88Hz), 6.10 (1H, d, J=6.20Hz), 3.90 (2H, br., s), 2.10 (3H, s); 13C NMR (100 MHz/CDCl3) δ ppm): 145.0, 127.0, 126.0, 124.5, 117.0, 115.5; Mass-ESI: 108.08 (M+H).

P-toludine(2h): IR (KBr pellet) $cm^{-1}$: 3418.79, 2914.50, 2859.65, 1515.51, 1268.63, 812.35;1HNMR (400 MHz/ CDCl3) δ ppm): 6.96 (2H, d, J=7.42Hz), 6.58 (2H, t, J=6.20Hz), 3.40 (2H, br., s), 2.25 (3H, s); 13CNMR (100 MHz/CDCl3) δ ppm): 143.7, 129.8, 127.5, 115.5, 20.5; Mass-ESI: 108.08 (M+H).

Benzene- 1, 4- diamine (2i): IR (KBr pellet) $cm^{-1}$ : 3410.54, 3075.80, 1526.80, 1096.43, 828.54; 1HNMR(400 MHz/ CDCl3) δ ppm): 6.50 (4H, s), 3.95 (2H, br., s);13C NMR (100 MHz/CDCl3) δ ppm) : 136.5, 138.0, 115.5; Mass-ESI: 109 (M+H).

## 3. Results and Discussion

### 3.1 UV-Visible studies of D-Glucosamine stabilized Pd nanoparticles

The $Pd^{2+}$ ion solution exhibits gradual change in color from transparent yellow to dark brown over time on addition of varying quantity of D-Glucosamine. The observed intense brown color suggests the formation of palladium nanoparticles and the reduction process is monitored using UV-visible spectroscopy. Figure 1 shows the broad continuous UV-visible spectra recorded for the sample prepared at room temperature compared with the absorbance peak of bulk palladium acetate solution. The characteristic absorption peak of palladium acetate at 400 nm vanished in the reaction progress, which revealed that bivalent palladium was reduced to zerovalent palladium completely in the end and the formation of the Pd nanoparticles. Thus the obtained spectra comparable with the earlier reports and reveal the complete reduction of bulk ions present in the precursor solution are converted to nanoparticles.

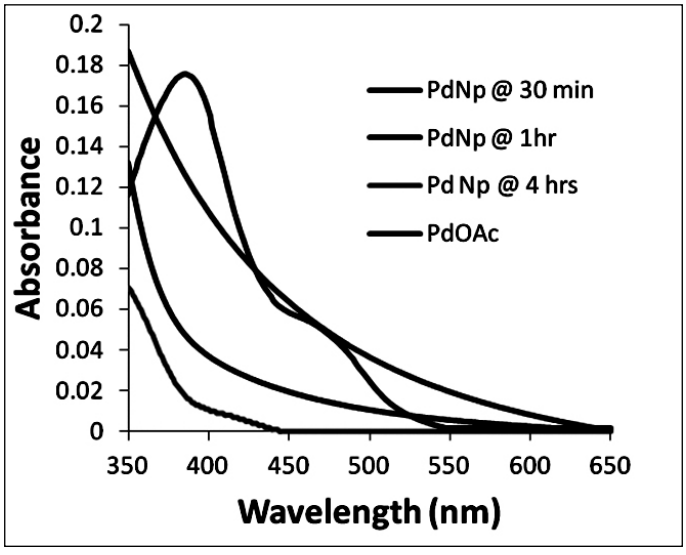



***Fig. 1:*** *UV-visible spectra of Palladium acetate and Pd NP using different amount of D-Glucosamine.*

### 3.2 XRD of D-Glucosamine stabilized Pd nanoparticles

The XRD patterns of D-Glucosamine stabilised Pd nanoparticles are presented in Figure 2. XRD pattern of D-Glucosamine stabilized Pd nanoparticles exhibits three sharp peaks at $2\theta = 40^{\circ}$, $46^{\circ}$ and $68^{\circ}$, which can be indexed as {111}, {200}, and {220} planes of a Pd metal with face centered-cubic (fcc) structure (JCPDS file No: 87-0638) respectively. It was found that as prepared Pd nanoparticles show good crystallinity. This indicates that PdNPs are mainly bound by (111) lattice plane, which has the lowest surface energy [36]. The average crystallite size was calculated for Pd NPs for its {111}, reflection from the line broadening using the Debye– Scherrer formula given by

$$D = k\lambda / \beta \cos\theta$$

where D is the average grain size, λ is the wavelength of the X-ray used (λ = 1.54Å for Cu Kα), θ is the Bragg angle (in degrees), β is the full width at half maximum after making the correction due to instrumental broadening and k is the shape factor. The value of k depends on several factors, including the Miller index of the reflecting plane and the shape of the crystals. If the shape is unknown, k is often assigned a value of 0.89. Calculated crystallite size for the as prepared D-Glucosamine stabilized Pd NPs was around 6 nm.

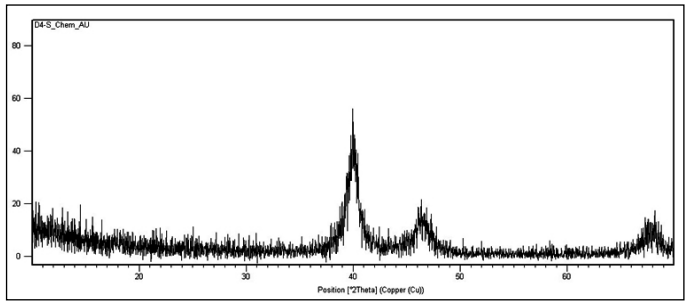



***Fig. 2:*** *Powder XRD patterns of D-Glucosamine stabilized Pd nanoparticles.*

### 3.3 TEM Studies of D-Glucosamine stabilized Pd nanoparticles:

TEM micrographs of as prepared D-Glucosamine stabilized Pd nanoparticles are presented in Fig 3. It clearly shows the spherical like morphology with average diameter ranging from 4 -5 nm. The particle size of D-Glucosamine stabilized Pd nanoparticles obtained from TEM was in good agreement with the average crystallite size calculated from the XRD data. This observation implies a rapid formation of small particles before aggregation and precipitation. This TEM observation is in good agreement with the visual observation where a rapid change of yellow solution to dark colloid was observed within 1 min before precipitation in the condition with a final pH of 10.

### 3.4 Catalytic Application in the Reduction of Nitro arenes

Further, the reduction of nitrobenzene to aniline by using nano $Pd^0$/Gluocosamine catalyst at R.T. Different substituted nitro benzenes were converted into their corresponding amines with good to moderate yields. It was observed from the literature that different palladium sources were used for this transformation and the details

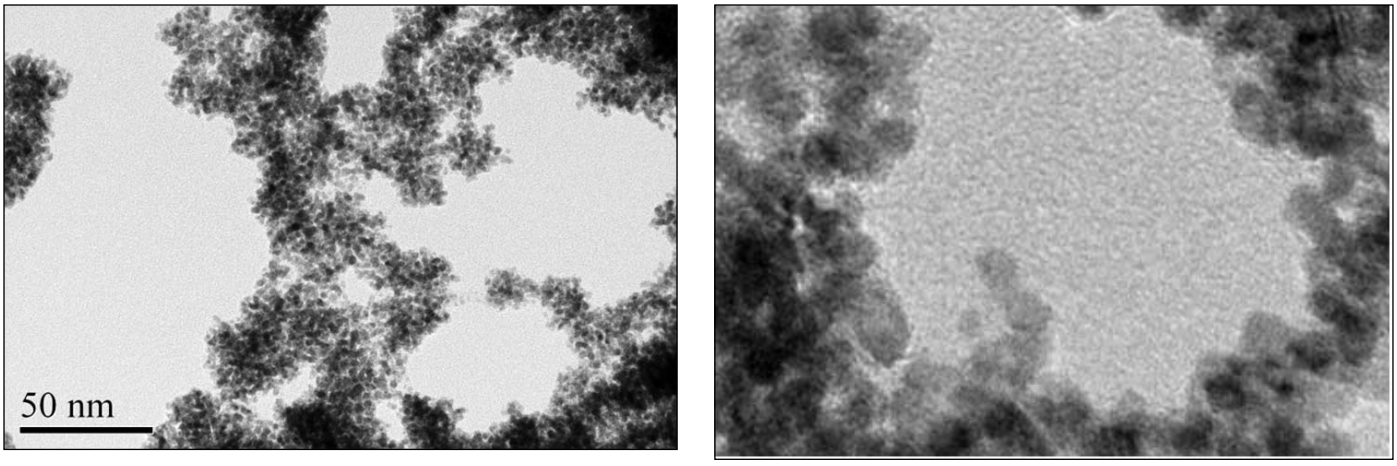
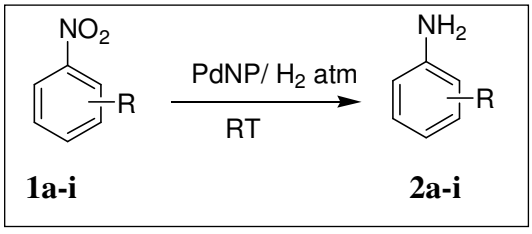



***Fig. 3:*** *RepresentativeTEM images of D-Glucosamine stabilized Pd nano particles*

are presented in Table-1. From Table-1, it was observed that the nano Pd/ Glucosamine resulted maximum yield of the desired product at lesser reaction times.




R = a) H b) 4-CHO c) 4-CN d) 4-Cl e) 4-$OCH_3$ f) 3-$CH_3$ g) 2-$CH_3$ h) 4-$CH_3$ i) $NH_2$

***Scheme–1:*** *Hydrogenation of nitrobenzene by using nano Pd NP.*

Initially to achieve the best optimized reaction conditions, reduction of nitrobenzene is taken as model reaction. Different concentrations of nitrobenzene, catalyst loading, and solvent effect were studied to define the optimum levels necessary to achieve efficient catalytic performance of Glucosamine stabilized Pd nanoparticles for the reaction described in (Scheme- 1).

**Table 1: Catalyst optimization**

| S.NO | Catalyst | Reaction Condition | Conversion (% ) | Yield[b] (% |
|---|---|---|---|---|
| 1 | Pd/C | 80ºC/1 hr | 100 | 34 |
| 2 | Pd/ZnO | 80ºC/1 hr | 100 | 19 |
| 3 | Pd/$CeO_2$ | 80ºC/1 hr | 100 | 90 |
| 4 | Pd/Sucrose [a] | RT/ 1hr | 100 | 98 |

[a] Reaction conditions: Nitrobenzene (1 mmol), $H_2$ atmosphere, solvent (2 mL) and 50 mg catalyst for 1 h. [b] Isolated Yields

The rate of conversion of nitrobenzene to aniline was investigated using different concentrations of nitrobenzene at 1, 1.5 and 2 mmol at room temperature using 50 mg catalyst for 60 min. It was found that 100 % conversion of the starting material was observed at 1 mmol concentration of nitrobenzene. Further it was observed that, at higher concentrations of nitrobenzene viz., 1.5 and 2 mmol, there was a decrease in the yield of final product.

Influence of various parameters such as temperature, solvents and catalyst loading were studied for the optimisation of the reaction conditions. The details are presented in the Table 2. Initially 50 mg of catalyst was used for optimizing the temperature and solvent. It was found that 96% of the product was formed at RT in 1h. Ethanol was found to be the best solvent after screening various solvents. It was also interesting to note that the 96% yield of the product was obtained in aqueous medium. The catalyst loading of 50 mg was sufficient to give maximum yield of the required product.

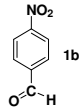
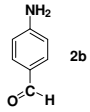
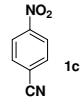
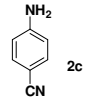
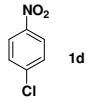
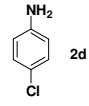
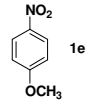
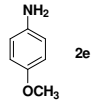
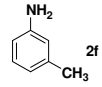
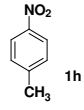
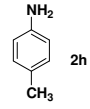
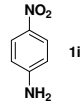
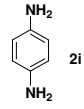
The effect of using different Pd NP concentrations on the catalytic hydrogenation of nitrobenzene is also listed in Table 2. From the data, it is observed that the % conversion was found to increase with an increase in catalyst weight. Only 25 % conversion of nitrobenzene was achieved using 5 mg catalyst, in comparison with 50 % obtained at a doubled weight of 10 mg catalyst. With a catalyst weight of 30 mg, the hydrogenation reaction reached 80%, whereas a full conversion of nitrobenzene to pure aniline with 100% selectivity was observed at 50 mg catalyst. It should be noted that controls using analogous samples without Pd showed no catalytic activity toward the conversion to aniline. These results indicated that the palladium nanoparticles stabilized in glucosamine could exhibit a remarkable catalytic performance for the reduction of nitrobenzene producing aniline with 100% yield.

**Table 2: Influence of temperature, solvent and catalyst loading**

| S.NO | Catalyst loading | Solvent | Temp in °C | Yield[b] (% ) |
|---|---|---|---|---|
| 1 | 50 | EtOH | RT | 96 |
| 2 | 50 | EtOH | 60 | 50 |
| 3 | 50 | EtOH | 90 | 30 |
| 4 | 50 | Water | RT | 80 |
| 5 | 50 | Toulene | RT | 70 |
| 6 | 50 | Isopropylalcohol | RT | 75 |
| 7 | 50 | EtOAc | RT | 20 |
| 8 | 50 | Hexane | RT | 15 |
| 9 | 50 | EtOh | RT | 80 |
| 10 | 20 | EtOH | RT | 70 |
| 11 | 10 | EtOH | RT | 50 |
| 12 | 5 | EtOH | RT | 25 |
| 13 | 0 | EtOH | RT | 0 |

As can be seen, under the present conditions, the freshly prepared PdNP catalyst is effective to completely convert 1 mmol nitrobenzene to aniline within 60 min at room temperature with 100% selectivity. By decreasing the reaction time, only 96% was converted obtained after 60 min. With further decrease in reaction time, a significant decrease was observed: The observed increase in nitrobenzene conversion% with time since the begining of the catalytic reaction clearly indicates that the Pd nanoparticles are well stabilized by carbohydrates (D-Glucosamine). To study the scope and applicability of the developed protocol, the reactions of various substituted nitrobenzenes were investigated. The results are shown in Table 3. Excellent yields of desired products were obtained with different substituted nitrobenzenes. The presence of electron withdrawing or electron donating groups at para position did not have any significant effect on the yield of the product. Hence the Pd/D-Glucosamine can efficiently catalyze the reduction of nitro aryls at RT by using ethanol as solvent. Yields were determined by comparision with authentic samples. Quantitative and qualitative analysis

**Table 3: Nano Pd/D-Glucosamine catalyzed reduction of different nitrobenzene**

| S.NO | Reactant | Product | Time (hrs) | Yield (%) |
|---|---|---|---|---|
| 1 |  1a |  2a | 1 | 96 |
| 2 |  1b |  2b | 2 | 86 |
| 3 |  1c |  2c | 2 | 89 |
| 4 |  1d |  2d | 1 | 92 |
| 5 |  1e |  2e | 2 | 85 |
| 6 |  1f |  2f | 2 | 90 |
| 7 |  1g |  2g | 2 | 92 |
| 8 |  1h |  2h | 1 | 95 |
| 9 |  1i |  2i | 2 | 86 |

of all amines were made by GC, GC–MS and identified by comparison with authentic samples. After completion of the reaction, the products were purified and recrystalized from methanol. These pure compounds were characterized by advance spectral techniques. In IR spectra, these compounds showed characteristic absorption peaks at a range 3300-3500 $cm^{-1}$ for N-H stretching in amines and 1100-1350 $cm^{-1}$ for C-N stretching. In $^1$HNMR spectral studies a chemical shift for $NH_2$ proton appear at δ 3.00-5.00

### 3.5 Catalyst Reusability

The recyclability of the catalyst is checked after completion of the reaction. The catalyst was separated by centrifugation, washed with water and subsequently with diethyl ether for three times. The catalyst was then dried at 120 °C and reused for next cycle. The reusability of the catalyst was checked for three cycles. It was found that the catalyst showed good recyclability for consecutive runs. The results were shown in Table- 4

**Table- 4: Recyclability of nano Pd/D-Glucosamine**

| Run | Fresh | Run 1 | Run 2 | Run 3 |
|---|---|---|---|---|
| % Yield[b] | 96 | 93 | 90 | 88 |

*[a] Reaction condition: 1 mmol Nitrobenzene, hydrogen atmosphere, 2ml ethanol,1 h at room temperature, [b] Isolated yield.*

## 4. Conclusion

We have prepared nano Pd/D-Glucosamine catalyst for catalytic transfer of hydrogenation by using hydrogen gas as hydrogen donor. The given methodology can also be applied at room temperature. The developed concept is expected to be more general and easily applicable. The Pd/D-Glucosamine catalyst is environmentally benign and can be used for reduction of nitro aryls to corresponding amines. This recyclable catalyst offers advantages like simple work-up and high yields. Hence the protocol fits into various green chemistry concepts such as use of nontoxic solvent, atom economy, ambient reaction temperature and recyclable, non-hazardous catalyst.

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# An efficient synthesis of pyridinone fused 1,3,4 – oxadiazole derivatives

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**Abstract**

A series of biaryl [1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one derivatives were synthesised from 4-bromo ethyl benzoate via a sequence of reactions hydrazide formation followed by oxadiazole synthesis, the oxadiazole was used in the synthesis of fused [1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one derivative. This was used in the synthesis of biaryl [1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one via Suzuki cross coupling reaction. All the compounds synthesised were characterized by $^{1}$H NMR, $^{13}$C NMR, and mass spectra.

## 1. Introduction

Heterocyclic chemistry is an important branch of organic chemistry accounting for nearly one-third of modern publications [1]. Heterocyclic compounds have vital role in our biological system. They are an integral part of many pharmacologically active molecules, natural products and nucleic acids. The base pair of DNA and RNA (guanine, cytosine, adenine and thymine) are also made up of heterocyclic compounds like purine, pyrimidine, etc. Synthesis of heterocyclic compounds has emerged as a powerful technique for generating new molecules useful for drug discovery [2]. In this review, our focus will be on the 3, 4-dihydropyrimidine (DHPM) ring. The pyrimidine is the most important heterocyclic moiety. Pyrimidine derivatives have been reported to exhibit antihypertensive [3], anticancer [4], antimicrobial [5], antihyperglycemic [6], antiarrhythmic, anti-inflammatory [7], analgesic [8], antibacterial [9], anti-HIV [10] and antitubercular activity [11]. Naturally occurring pyrimidines are of great interest due to their diverse pharmacological properties, and this attracts attention of many medicinal chemists to further derivatization of the heterocycle backbone and screening the resulting compounds for their biological activity.

1,3,4 - Oxadiazoles possess a large number of chemical and variety of biological properties.1,3,4-oxadiazoles were permit to increase their biological activities due to H-bonding with receptors. These derivatives possess a variety of biological activities which include anticancer [12-15], antiviral [16], antifungal [17], antibacterial [18], anti-inflammatory [19], anti-anxiety [20], antitubercular [21] and antidepressant [22]. The Zibotentanwas a 1,3,4-oxadiazole nucleus containing most important anticancer drug available in the market.

## 2. Experimental

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA) and Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. $^{1}$H and $^{13}$C NMR spectra were recorded on Gemini Varian-VXR-unity (300 MHz and 75 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. CHN analysis was carried out using Vario Micro Cube Elemental analyzer, Germany. Melting points were determined with an electro-thermal melting point apparatus, and are uncorrected.

**5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine (3):** To a stirred solution of ethyl 4-bromobenzoate **I** (50 g, 0.218 mol) in ethanol (750 mL) was added hydrazine hydrate (50 mL, 1 vol) and stirred at reflux temp for 16 h. The solvent was evaporated, the residue was triturated with pet ether to give hydrazide. The hydrazide was taken in ethanol (500 mL) and added cyanogen bromide (34 g, 0.327 mmol) and stirred at RT for 16 h. After completion, the solvent was evaporated and the residue was poured into ice water and filtered the solid to obtain oxadiazole **1** (37.7 g, 72%, over two steps) as a white solid; m. p. 270-272 °C;IR (KBr) υ: 3292, 3110, 1660 cm$^{-1}$; $^{1}$H NMR (DMSO-$d_6$, 300 MHz): δ 7.75 (t, 4H, Phenyl), 7.35 (s, 2H, -$NH_2$); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 163.7, 156.6, 132.3, 126.9, 123.7, 123.4; [Lit-23] $^{1}$H NMR ($CDCl_3$): δ 7.25-7.69 (dd, 4H, J = 2.6, 5.6 Hz, Ar-H), 7.75 (s, 2H, NH); $^{13}$C NMR ($CDCl_3$): 144-130 (Ar-C). LC-MS: m/z 240.09 (M+H). HRMS. Calcd. for $C_8H_6BrN_3O$: 240.0875, Found: 240.0879.

**2-(4-bromophenyl)-7-hydroxy-5H-[1,3,4] oxadiazolo[3,2-a] pyrimidin-5-one (4):** To a stirred solution of 5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine (**1**) (20 g, 83.2 mmol) in chlorobenzene (400 mL) was added bis(1,3,5,trichlorophenyl)malonate (38.4 g, 83.2 mmol) at

100 °C and the mixture was refluxed under stirring for 1 h. The reaction mixture was cooled and diluted with pet ether, solid that precipitated was filtered, washed with pet ether to give pyrimidinone **2** (23.6, 92%) as a pale yellow solid; m. p. 290-292 °C; IR (KBr) υ: 3447, 3087, 2923 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 12.03 (s, 1H, -OH), 7.98-7.87 (m, 4H, Phenyl), 5.36 (s, 1H, pyrimidine); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 169.3, 156.9, 156.1, 155.2,132.7, 128.7, 127.1, 121.4, 84.7; LC-MS: m/z 308.4 (M+H). HRMS. Calcd. for $C_{11}H_6BrN_3O_3$: 308.4012, Found: 308.4017.

**General procedure for Suzuki coupling:** A suspension of 2-(4-bromophenyl)-7-chloro-5H-[1,3,4] oxadiazolo[3,2-a] pyrimidin-5-one**3** (1 mmol), $ArB(OH)_2$ (1.2 mmol), $Na_2CO_3$ (2.5 mmol) in DCE (8 vol) and $H_2O$ (2 vol) was degassed for 30 min, then added $Pd(PPh_3)_4$ (0.05 mmol) and refluxed under stirring for 16 h. The reaction mixture was cooled and poured into ice water (500 mL), solid that precipitated was filtered, washed with diethyl ether to give desired product **5**.

**2-(4′-chloro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5a):** m. p. 212-214 °C; IR (KBr) υ: 3437, 3088, 2921 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.04 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 8.1 Hz, 2H), 7.82 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 4.24 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 172.1, 157.3, 155.9, 155.5, 138.9, 133.2, 132.2, 129.3, 128.5, 127.4, 126.5, 120.9, 86.2; LC-MS: m/z 340.7 (M+H).

**2-(2′,4′-difluoro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5b):** m. p. 225-227 °C; IR (KBr) υ: 3443, 3092, 2938 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.11 (d, J = 8.1 Hz, 2H), 7.78 (d, J = 8.1 Hz, 2H), 7.77-7.69 (m, 1H), 7.43-7.51 (m, 1H), 7.25-7.33 (m, 1H), 4.25 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 169.5, 157.8, 155.4, 154.9, 147.8, 143.2, 138.8, 131.2, 129.9, 129.1, 127.6, 126.9, 126.2, 121.2, 85.9; LC-MS: m/z 342.1 (M+H).

**2-(3′-fluoro-5′-methoxy-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5c):** m. p. 193-195 °C; IR (KBr) υ: 3452, 3068, 2903 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.09-7.95 (m, 4H), 7.25-7.15 (m, 2H), 6.90 (d, J = 8.1 Hz, 1H), 4.26 (s, 1H), 3.87 (s, 3H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 171.2, 159.6, 157.2, 155.6, 152.3, 148.5, 137.2, 130.7, 130.1, 129.5, 127.9, 126.9, 126.2, 122.1, 86.5, 65.2; LC-MS: m/z 354.2 (M+H).

**2-(3′-chloro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5d):** m. p. 210-212 °C; IR (KBr) υ: 3437, 3088, 2921 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.06-7.94 (m, 4H), 7.65-7.45 (m, 3H), 7.25 (t, J = 6.3 Hz, 1H), 4.25 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 169.2, 159.9, 156.8, 155.1, 137.4, 131.7, 130.9, 129.7, 128.5, 127.3, 126.5, 124.2, 120.1, 118.3, 86.5; LC-MS: m/z 340.7 (M+H).

**2-(2′-fluoro-3′-methoxy-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5e):** m. p. 251-253 °C; IR (KBr) υ: 3431, 3082, 2929 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.02 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.29-7.08 (m, 3H), 4.24 (s, 1H), 3.90 (s, 3H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz); δ 170.9, 159.1, 156.9, 156.1, 152.1, 148.3, 136.4, 130.6, 130.1, 129.5, 127.8, 126.4, 125.6, 122.1, 86.1, 61.4; LC-MS: m/z 351.9 (M+H).

**2-(2′-fluoro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5f):** m. p. 195-197 °C; IR (KBr) υ: 3441, 3091, 2918 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.05 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.4 Hz, 2H), 7.29-7.08 (m, 4H), 4.25 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 171.6, 156.9, 155.8, 154.1, 138.4, 133.1, 132.1, 129.1, 128.7, 127.4, 126.4, 120.8, 85.5; LC-MS: m/z 324.2 (M+H).

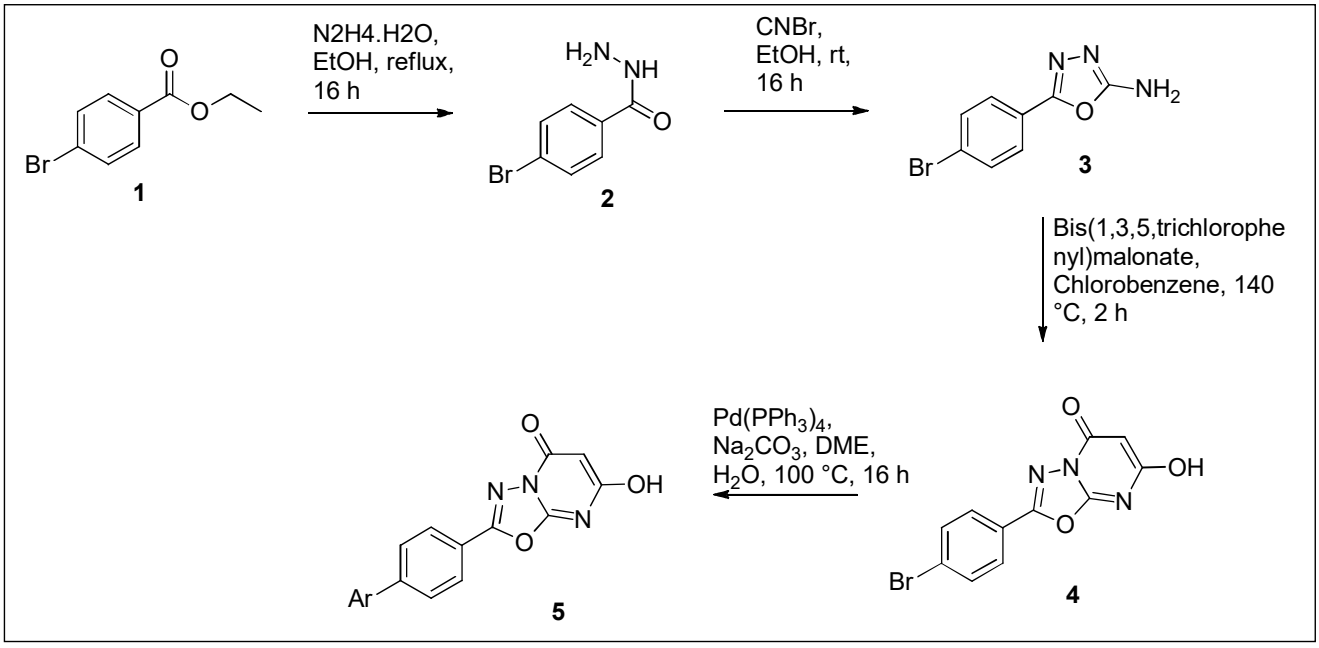
**2-(3′-fluoro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5g):** m. p. 167-169 °C; IR (KBr) υ: 3448, 3094, 2927 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.06-7.94 (m, 4H), 7.65-7.45 (m, 3H), 7.25 (t, J = 6.3 Hz, 1H), 4.25 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 169.2, 160.2, 156.8, 155.1, 137.4, 131.7, 130.9, 129.7, 128.5, 127.3, 126.5, 124.2, 120.1, 118.2, 86.4; LC-MS: m/z 321.9 (M+H).

**7-hydroxy-2-(3′,4′,5′-trifluoro-[1,1′-biphenyl]-4-yl)-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5h):** m. p. 271-273 °C; IR (KBr) υ: 3448, 3091, 2926 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.08-7.98 (m, 4H), 7.89-7.81 (m, 2H), 4.28 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 172.1, 159.2, 156.7, 155.8, 139.5, 134.2, 132.1, 129.3, 128.5, 127.4, 120.9, 85.6; LC-MS: m/z 358.0 (M-H).

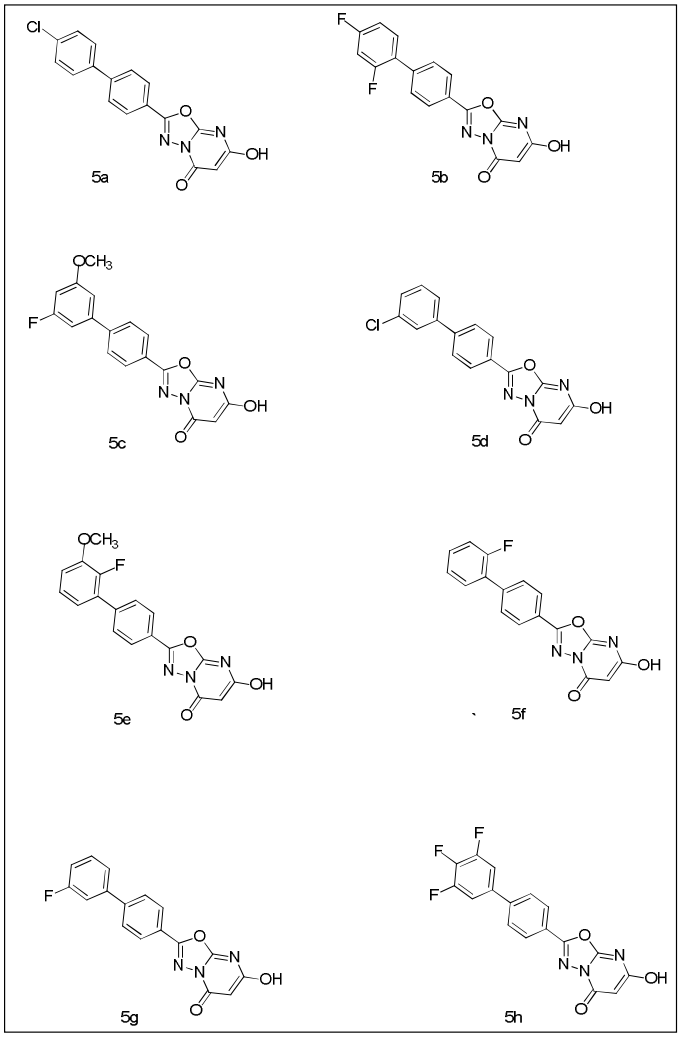
**2-(4′,5′-difluoro-2′-methoxy-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5i):** m. p. 237-239 °C; IR (KBr) υ: 3440, 3081, 2919 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.97 (d, J = 7.8 Hz, 2H), 7.72 (d, J = 7.8 Hz, 2H), 7.59-7.53 (m, 1H), 7.38-7.32 (m, 2H), 4.27 (s, 1H), 3.82 (s, 3H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 172.5, 159.4, 156.8, 155.1, 154.3, 138.7, 134.3, 132.1, 129.4, 128.5, 127.4, 120.9, 85.6, 66.2; LC-MS: m/z 372.2 (M+H).

**2-(3′,5′-dichloro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5j):** m. p. 251-253 °C; IR (KBr) υ: 3446, 3093, 2927 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): δ 8.06-8.00 (m, 4H), 7.95 (s, 2H), 7.71 (s, 1H), 4.30 (s, 1H); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): δ 169.2, 156.9, 155.6, 155.2, 138.9, 132.9, 132.4, 129.1, 128.4, 127.3, 126.4, 120.7, 86.5; LC-MS: m/z 374.4 (M+H).

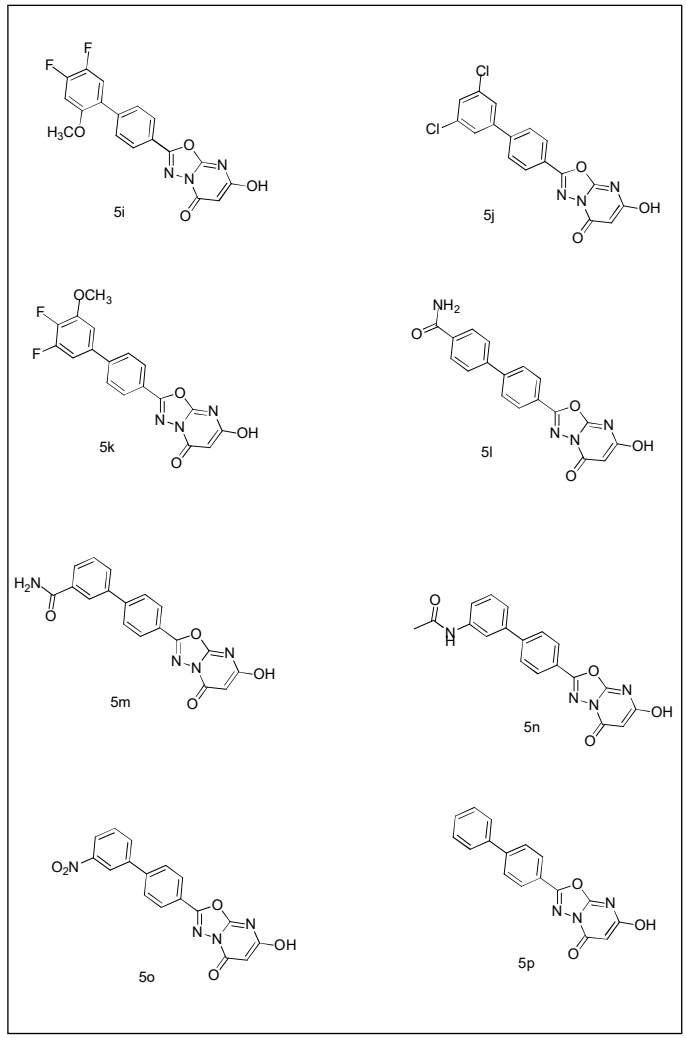
**2-(3′,4′-difluoro-5′-methoxy-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (5k):** m. p. 224-226 °C; IR (KBr) υ: 3446, 3075, 2918 $cm^{-1}$; $^1H$




**Scheme-1:** *Synthetic scheme for the preparation of fused pyridone 1,3,4-oxadiazoles*




**Scheme-2**




**Scheme-3**

NMR (DMSO-$d_6$, 300 MHz): δ 8.05-7.95 (m, 4H), 7.51-7.35 (m, 2H), 4.30 (s, 1H), 4.02 (s, 3H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 171.9, 156.2, 155.2, 154.6, 153.1, 138.9, 133.2, 132.2, 129.3, 128.5, 127.1, 126.4, 120.9, 86.1, 65.2; LC-MS: m/z 372.2 (M+H).

**4′-(7-hydroxy-5-oxo-5H-[1,3,4]oxadiazolo[3,2-a] pyrimidin-2-yl)-[1,1′-biphenyl]-4-carboxamide (5l):** m. p. 192-194 °C; IR (KBr) υ: 3450, 3091, 2916 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 8.13-7.94 (m, 7H), 7.93-7.83 (m, 2H), 7.41 (s, 1H), 4.31 (s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 172.4, 156.1, 155.9, 155.1, 153.7, 138.2, 133.7, 132.6, 129.7, 128.2, 127.1, 126.6, 121.2, 85.5; LC-MS: m/z 349.0 (M+H).

**4′-(7-hydroxy-5-oxo-5H-[1,3,4]oxadiazolo[3,2-a] pyrimidin-2-yl)-[1,1′-biphenyl]-3-carboxamide (5m):** m. p. 220-222 °C; IR (KBr) υ: 3450, 3090, 2916 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 8.32 (s, 1H), 8.15 (s, 1H), 8.12-7.91 (m, 6H), 7.62-7.41 (m, 2H), 4.41 (s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 172.4, 156.1, 155.9, 155.1, 153.7, 138.2, 133.7, 132.6, 129.7, 128.2, 127.1, 126.6, 121.2, 85.5; LC-MS: m/z 348.9 (M+H).

**N-(4′-(7-hydroxy-5-oxo-5H-[1,3,4]oxadiazolo[3,2-a] pyrimidin-2-yl)-[1,1′-biphenyl]-3-yl)acetamide (5n):** m. p. 175-177 °C; IR (KBr) υ: 3449, 3083, 2913 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 10.2 (s, 1H), 8.07-7.82 (m, 3H), 7.64 (s, 1H), 7.45-7.37 (m, 2H), 4.26 (s, 1H), 2.08 (s, 3H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 171.9, 156.2, 155.2, 154.6, 153.1, 138.9, 133.2, 132.2, 129.3, 128.5, 127.1, 126.4, 120.9, 86.1, 30.2; LC-MS: m/z 363.0 (M+H).

**7-hydroxy-2-(3′-nitro-[1,1′-biphenyl]-4-yl)-5H-[1,3,4] oxadiazolo[3,2-a]pyrimidin-5-one (5o):** m. p. 235-237 °C; IR (KBr) υ: 3432, 3091, 2931 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 8.56 (s, 1H), 8.35-8.28 (m, 2H), 8.12-8.05 (m, 4H), 7.86-7.78 (m, 2H), 4.32 (s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 171.6, 156.5, 155.6, 154.1, 138.3, 133.6, 132.1, 129.1, 128.4, 127.4, 126.4, 120.8, 86.2; LC-MS: m/z 348.9 (M+H).

**2-([1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4] oxadiazolo[3,2-a]pyrimidin-5-one (5p):** m. p. 175-177 °C; IR (KBr) υ: 3442, 3086, 2919 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 8.05-7.31 (m, 9H), 4.45 (s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 174.2, 157.1, 155.7, 155.1, 138.5, 132.1, 131.3, 128.4, 127.3, 126.4, 120.8, 84.2; LC-MS: m/z 304.0 (M-H).

## 3. Results and Discussion

The general synthesis of oxadiazolo [3, 2-a] pyrimidin-5-one is given in scheme 1 and various derivatives synthesized are shown in Schemes 2 and 3. The compound (ethyl 4-bromobenzoate) **1** reacted with hydrazine hydrate in ethanol at 80 °C to give compound **2** (4-bromobenzohydrazide) which on treatment with CNBr in EtOH at room temperature yields compound **3** (5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine). The compound **3** on reaction with bis(1,3,5-trichlorophenyl) malonate in chlorobenzene at 140°C for 2 h gave the compound **4,** which was converted into final compound **5** (7-substituted phenyl-2-(4-chlorophenyl)-5H-[1, 3, 4] oxadiazolo [3, 2-a] pyrimidin-5-one) via Suzuki mediated cross coupling using corresponding aryl boronic acid, $Pd(PPh_3)_4$, $Na_2CO_3$ in DME at 100°C for 16 h. These final compounds (**5a-p**) were characterized by NMR and mass spectra. IR spectra showed strong absorption band at 3354 cm$^{-1}$ due to alcohol group. The characteristic signals in $^1$H NMR of 2-(2′,4′-difluoro-[1,1′-biphenyl]-4-yl)-7-hydroxy-5H-[1,3,4] oxadiazolo[3,2-a]pyrimidin-5-one (**5b**) of the pyridone proton displayed as singlet at 4.25 ppm confirms the presence of fused pyridone system. The mass spectrum of **5b** showed a molecular ion peak at m/z = 342 $(M+H)^+$ corresponding to a molecular formula $C_{17}H_9F_2N_3O_3$.

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