PHYSICAL CHARACTERIZATION WITH SYNTHESIS AND DYNAMIC ANTIBACTERIAL ACTIVITY OF PEGYLATED COUMARIN DOPED BY SILVER AND GOLD NANOPARTICLES

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ABSTRACT:

Drug–polymer conjoin is a simple and efficient approach to synthesizing new, effective, dynamic antibacterial agents to counter the problem of microbial resistance. PEGylated coumarins were synthesized via PEGylation process. The resultant series of PEGylated coumarin were characterized by various techniques such as FTIR, 1HNMR, 13CNMR, Mass spectral data and elemental analysis. These compounds were used for surface activation on to synthesized gold/silver nanoparticles. The noncovalent molecular interactions amongst the metallic nanoparticles with PEGylated coumarin suggested by TEM images of surface functionalized AgNPs and AuNPs PEGylated coumarin and UV analysis. Antimicrobial activities of the new synthesis compounds were investigated against Gram-positive bacteria Staphylococcus aureus, Gram-negative bacteria Escherichia coli, by cup plate method. Antibacterial activity was studied in terms of inhibition zone diameters, in addition to the estimation of minimal inhibitory concentration (MIC) (100, 50 and 25 μg/mL) of the prepared compounds. E. coli and S.aureus were the most affected microorganisms among the tested microorganisms with an inhibition zone of 5–32 (mm) in case of Pegylated coumarin and 5-31(mm) inhibition zone of Nanoparticles containing pegylated coumarins. The obtained results showed that the all The newly synthesized compounds have higher, randomly and potent activity with all MIC concentrations.

Keywords: PEGylated Coumarins, silver and gold Nanoparticles, antibacterial activity.

1. INTRODUCTION

Coumarins are a vital category of common oxygen hetero-cyclic composites. The extensive scope of biological activities pertaining to coumarins and their therapeutic impact against various pathologies have received significant attention in developing several drugs [1, 2, 3, 4, 5]. Furthermore, medicinal chemists have been fascinated by the stability and solubility of these compounds which has led to their medicinal applicability [6,7]. In the combination of coumarin moieties with poly(ethylene glycols) (PEGs), PEG[8] in its most common form is a linear or branched polyether concluded with hydroxyl groups. PEG is synthesized by anionic polymerization of ethylene oxide initiated by nucleophilic attack of a hydroxide ion on the epoxide ring. Most useful for polypeptide alteration is monomethoxy PEG (mPEG). On the other hand, mPEG is synthesized by anionic ring opening polymerization started with methoxide ions. Successful conjugation of PEG with biomolecule depends upon the chemical structure, molecular weight, steric hindrance, and the reactivity of the biomolecule as well as the polymer. In order to synthesize a bioconjugate, both chemical entities (i.e., the bioactive as well as the polymer) required to possess a reactive or functional group such as –COOH, –OH, –SH, or –NH2. These PEGs have unique properties, including solubility in broad range of solvents, lack of toxicity, lack of antigenicity and immunogenicity, non-interference with enzymatic activities thus making them ideal combination for further applications as drug discovery and drug delivery agents. Due to these qualities, PEG chemistry has shown broad based applications, which may be in large part ascribed to the use of PEG-conjugates to deliver drugs, oligonucleotides or enzymes.[9] It is well known, for example, that the use of coumarins in the phototherapy of many skin diseases, is due to their capability to undergo electronic transitions when UVA-irradiated, working as photosensitizer drugs.[10,11] The PEGylated 4-methyl- and 4, 8-dimethylcoumarins have shown an enhanced and improved capability to inhibit TNF-a induced ICAM-1 expression on human endothelial cells as compared to the monomeric analogues. The additional benefits of synthesized PEGylated co-polymers is their enhanced
solubility in aqueous and organic media, thus making them suitable for bio-medical preparations and applications.\[12\]

The nanomaterial industry generates gigantic quantities of metal-based nanomaterials for various technological and biomedical applications.\[13\] In recent years, there has been a growing interest in the synthesis of metal nanoparticles (MNP)s such as silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) due to their useful properties for applications in different areas of medicine, biology, catalysis, and antibacterial\[14,15,16,17\]. Metallic nanoparticles such as silver nanoparticles (AgNPs) have gained lots of attention due to the continuous upsurge in microbial infections and diseases, and also inefficient treatment. Also, due to rapid intensification in the antibiotic resistance in this period has revived the consideration of the researchers and scientists to explore the therapeutic abilities of silver and its nanoparticle systems as potential antimicrobial agents.\[18\] similarly gold nanoparticles (AuNPs) have tremendous potential for bionanotechnology-based applications, thus resulting in new possibilities and insights within the medical research field. These nanoparticles can be used for selective transportation mechanism through receptor ligand binding. During various synthesis processes of AuNPs, several difficulties have been encountered. Therefore, the scientific research community turned its attention towards tuning and perfecting the obtaining approaches involved in AuNPs synthesis.\[19\] A surface modification was applied on nanoparticles AuNPs and AgNPs. An exceptional characteristic of coumarin as fluorescent probes and triplet sensitizers and surface passivation on nanoparticle chemistry have attracted our attention to synthesize PEGylated-coumarins and evaluation of their applications in rapid advances in nanotechnology.

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. Microbial pathogens in food may cause spoilage and contribute to foodborne disease incidence, and the emergence of multidrug resistant and disinfectant resistant bacteria—such as Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), and Pseudomonas aeruginosa (P. aeruginosa)—has increased rapidly, causing the increase of morbidity and mortality.\[20\] The advancement of new benign microbial strains to the present anti-infective agents has been a major issue in common health systems; subsequently, there is a perquisite to develop novel bactericidal agents. The microbial strains have been reported to exist with varied film assemblies which allows to recognize them as Gram-negative (G-ve) or Gram positive (G+ve).\[18\]

PEGylated coumarins compounds were used for surface activation on to synthesized gold/silver nanoparticles. The antibacterial activity of the prepared compounds has been investigated. As the compounds exhibited momentous in vitro antibacterial against clinical isolates of Gram-positive bacteria Staphylococcus aureus, Gram-negative bacteria E. coli.

II. EXPERIMENTAL WORK

2.1 Material and methods

Chloroauric acid (HAuCl₄.4H₂O) was obtained from Acros Organics (New Jersey, USA), Silver nitrate (AgNO₃), NaBH₄, trisodium citrate were procured from Sigma-Aldrich (St. Louis, MO, USA), PEG 200 was obtained from SD-Fine chemicals Ltd. (Mumbai, India), all other reagents required for the synthesis of the nanoparticles conjugated PEGylated coumarins were of synthesis grade and purchased from Acros Organics, Sigma-Aldrich, Qualigens and SD-Fine Chemicals. All solvents were distilled prior to use. Water purified by a Millipore system was used for making the solutions. Thin-layer chromatography was performed on silica gel G. Melting points were determined by the open capillary method and are uncorrected. Absorption measurements were carried out on Shimadzu UV-1700 Pharma Spec. The FT-IR measurements for samples were carried out using KBr pallets on Shimadzu FT-IR spectrophotometer. The 1H NMR spectra and 13CNMR spectra were recorded in DMSOD6/CDC13 on a Bruker Avance II 400 NMR spectrometer. Chemical shifts are reported using TMS as an internal standard. Mass spectra were recorded using a Shimadzu gas chromatograph. Elemental analyses were performed on a Perkin Elmer 2400 instrument. Samples for transmission electron microscope (TEM) were prepared by putting a drop of the colloidal solution on a copper grid coated with a thin amorphous carbon film. Samples were dried and kept under vacuum in desiccators before putting them in a specimen holder. TEM characterization was carried out using a Philips CM-200 electron microscope. Particle size was measured from the TEM micrographs. The particle size was calculated by taking average of at least 100 particles.

2.2. General Procedure

2.2.1 Preparation of 7-hydroxy-4-methyl-2H-chromen-2-one(2a):

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Resorcinol (0.1 mmol) and ethyl aceto acetate (0.1 mmol) were dissolved in H₂SO₄ (75% 20 ml) mixture was stirred well and kept overnight. It was diluted with ice cold water. The solid separate was crystallized from dilute ethanol to obtain the 7-hydroxy-4-methyl-2H-chromen-2-one (3a).

**Scheme 2** Molecular Formula: C₁₀H₈O₃, Yield: 82%, Melting point: 186°C, IR (KBr/λ_max cm⁻¹): 3501 (-OH), 1671 (-C=O), ¹H NMR (400 MHz, CDCl₃/DMSO-d₆): δ(ppm) 2.36 (s, 3H, -CH₃), 6.08-6.09 (d, 1H, J=5.04, Ar-H), 6.68-6.69 (d, 1H, J=2.32, Ar-H), 6.78-6.80 (q, 1H, Ar-H), 7.53-7.55 (d, 1H, J=8.72, Ar-H), 10.58 (s, 1Hf, -OH), ¹³C NMR (200 MHz CDCl₃) δ 18.06, 102.12, 110.19, 111.91, 112.75, 126.25, 153.19, 154.77, 160.24, 161.09; Mass Spectrum: m/z 176 M⁺, CHN calculated: C 68.18, H 4.58 CHN found: C 68.21, H 4.54

**Scheme 1: Synthesis of 7-hydroxy-4-methyl-2H-chromen-2-one**

**2.2.2. Preparation of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid (3b):**

Anhydrous Citric acid (10gm) was heated with conc. H₂SO₄ (30ml) on a water bath till the evaluation of carbon monoxide gas ceased. After cooling, m-Cresol (0.04 mol, 4.32 gm, 4.18 ml) was added followed by conc. H₂SO₄ (10ml). The reaction mixture was kept for 24 hrs at room temperature. It was then poured in to ice cold water and solid separted was crystallized from dilute ethanol to get 7-methyl coumarin-4-acetic acid (3b).

**Scheme 3** Molecular Formula: C₁₁H₈O₅, Yield: 72%, Melting point: 206°C, IR (KBr/λ_max cm⁻¹): 3499 (-OH), 1614 (-C=O), ¹H NMR (400 MHz, CDCl₃/DMSO-d₆): δ(ppm) 2.51 (s, 3H, -CH₂), 6.22 (s, 1Hd, Ar-H), 6.73-6.82 (m, 1Hb, Ar-H), 7.51-7.54 (d, 1Ha, J=8.76, Ar-H), 10.59 (s, 1Hg, -OH), 12.77 (s, 1Hf, -COOH), ¹³C NMR (200 MHz CDCl₃) δ 37.22, 102.27, 111.34, 111.91, 112.97, 126.67, 150.12, 154.99, 160.22, 161.15, 170.64; Mass Spectrum: m/z 220 M⁺, CHN calculated: C 60.00, H 3.66 CHN found: C 68.21, H 4.54

**Scheme 2: Synthesis of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid**

**2.2.3 Preparation of 7-hydroxy-4-methyl-8-nitro-2H-chromen-2-one (3c):**

The nitration of 7-hydroxy-4-methylcoumarin using concentrated nitric acid and sulphuric acid at 5°C gave two nitro isomers i.e. 7-hydroxy-4-methyl-8-nitrocoumarin & 7-hydroxy-4-methyl-6-nitrocoumarin. In a conical flask 7-hydroxy-4-methyl coumarin (12 gm) was dissolved in conc. H₂SO₄ acid (100 ml) and was then kept in an ice bath. When the temperature inside the flask is below 1°C, 20 ml of nitrating mixture (5ml of concentrated nitric acid and 15 ml of concentrated sulphuric acid) taking care that the temperature does not rise above 10°C. After the addition was completed, the flask was removed from the ice bath and kept at room temperature for an hour. The flask was shaked occasionally during this period and then poured with stirring in a beaker containing crushed ice. The crude product was filtered which is a mixture of 6 and 8 nitro derivatives and washed with cold water. The crude mixture was transferred in a conical flask containing ethanol and boiled. The residue is 6-nitro-4-methyl-7-hydroxy coumarin, the filtrate, was cooled in an ice bath, 8-nitro derivative soon crystallized out. Recrystallized from ethanol and 8-nitro-4-methyl-7-hydroxy coumarin was collected (3c).

**Scheme 4** Molecular Formula: C₁₀H₇NO₅, Yield: 88%, Melting point: 255°C, IR (KBr/λ_max cm⁻¹): 3507 (-OH), 1671 (-C=O), ¹H NMR (400 MHz, CDCl₃/DMSO-d₆): δ(ppm) 2.50 (s, 3He, -CH₃), 6.19-6.18 (d, 1H, J=2.36, Ar-H), 7.21-7.23 (q, 1H, Ar-H), 8.17-8.20 (d, 1H, J=8.72, Ar-H), 10.28 (s, 1H, -OH), ¹³C NMR (200 MHz CDCl₃) δ 18.24, 112.29, 114.30, 116.20, 128.99, 132.72, 147.52, 152.02, 160.99; Mass Spectrum: m/z 221 M⁺, CHN calculated: C 54.31, H 3.19, N 6.33; CHN found: C 54.32, H 3.24, N 6.36.
2.2.4 Preparation of 7-hydroxy-8-ido-4-methyl-2H-chromen-2-one (3d):

To 7-hydroxy-4-methyl coumarin (8.8 gm, 50 mmol) in ethanol, a mixture of I₂ (4.5 gm) and HIO₄ (2.5 gm) in ethanol (50 ml) was added. The solution was stirred at room temperature for 4 hrs and poured onto ice cold water. Solid obtained was filtered and crystalline from ethanol. Molecular Formula: C₁₀H₇I₂O₃, Yield: 70%, Melting point: 163°C, IR (KBr/λmax cm⁻¹): 3503 (-OH), 1679 (-C=O), ¹H NMR (400 MHz, CDCl₃/DMSO-d₆): δ(ppm) 2.45 (s, 3H, -CH₂), 6.12 (s, 1H, Ar-H), 6.62-6.63 (d, 1H, J=2.4, Ar-H), 7.69 (s, 1H, Ar-H), 10.13 (s, 1H, -OH), ¹³C NMR (200 MHz, CDCl₃) δ 19.00, 84.34, 110.79, 112.37, 115.99, 135.55, 152.49, 153.79, 156.66, 160.74., Mass Spectrum: m/z 302 M⁺, CHN calculated: C 39.76, H 2.34  CHN found: C 39.78, H 2.25

2.2.5 Preparation of 3-bromo-7-hydroxy-4-methyl-2H-chromen-2-one (3e):

Take 7-hydroxy-4-methyl coumarin (3.52 gm, 20 mmol) in acetic acid (30 ml), Br₂ (1.1 ml, 20 mmol) in acetic acid (20 ml) was added with stirring and the solution was stirred further at room temperature for 2 hr solution was filtered and solid was washed with water crystallized from ethanol to obtained product C₁₁H₈O₅, Yield: 72%, Melting point: 206°C, IR (KBr/λmax cm⁻¹): 3501 (-OH), 1671 (-C=O), ¹H NMR (400 MHz, CDCl₃/DMSO-d₆): δ(ppm) 2.69 (s, 3H, -CH₃), 6.37 (s, 1H, Ar-H), 6.88-6.89(d, 1H, J=2.4, Ar-H), 8.10 (s, 1H, Ar-H), 10.39 (s, 1H, -OH), ¹³C NMR (200 MHz, CDCl₃) δ 19.01, 111.00, 112.99, 113.85, 116.75, 132.00, 148.09, 153.89, 155.20, 161.29; Mass Spectrum: m/z 253 M⁺, CHN calculated: C 60.00, H 3.66 CHN found: C 68.21, H 4.54

Table 1: Physical characterisation data of substituted coumarin derivatives (3a-e)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>Mol. Weight</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>Mol. Formula</th>
</tr>
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<tr>
<td>3a</td>
<td></td>
<td>176</td>
<td>82%</td>
<td>186°C</td>
<td>C₁₀H₆O₃</td>
</tr>
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</table>
2.2.6. Synthesis of hydroxycarboxy poly ethylene glycol (HO-PEG\textsubscript{200}COOH)

Poly ethylene glycol (28.0 mmol, 5mL) was dissolved in 20 mL of dry CH\textsubscript{2}Cl\textsubscript{2}. To this solution was added THF containing maleic anhydride (56.0 mmol, 0.54 mg) and pyridine (56.0 mmol, 0.46 mL). The mono acid derivative of poly(ethylene glycol)\textsubscript{200} was used without purification shown in Scheme 7.

2.2.7. Synthesis of Oxy-Terminal PEGylated 7-hydroxy-4-methyl-2H-chromen-2-one (4a):

The mono acid derivative of carboxohydroxy PEG (HO-PEG\textsubscript{200} COOH) (28.0 mmol) was activated with 1:2 molar equivalent of 7-hydroxy-4-methyl-2H-chromen-2-one (4a) (46.0 mmol). N,N dicyclocarbidiimide (46.0 mmol) was dissolved in dichloromethane. (Scheme 8) The solution was stirred for 24 h at room temperature. A syrpy resin obtained was dried under vacuum. The resin was dissolved in 15mL of acetone. A white precipitate of dicyclohexylurea (DCU) appeared was discarded and filtrate was collected. The final filtrate was evaporated to afford the product. TLC in (ethyl acetate: methanol 8:2) was performed to check the presence of DCU. It showed a negative result. The resin was dried in vacuum for IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and Mass characterization. At this stage the resin did not stick anymore to the glass wall. IR spectrum of resin showed the characteristic absorption band PEG ether backbone (1103 cm\textsuperscript{-1}) and absorption bands 1702 cm\textsuperscript{-1} for the ester, Yield : 93%, density: 1.043cm\textsuperscript{3}, IR (KBr\textsubscript{\text{max},cm\textsuperscript{-1}}) 3334(OH-PEG); 2928 (CH\textsubscript{2}-PEG), 2872(-CH3); 1702(-C=O, -PEG), 1103(-CH\textsubscript{2}-O-).
CH₂) cm⁻¹, ¹H NMR (400 MHz,CDCl₃/DMSO-d₆) □ (ppm) 1.02 (m, OH-PEG Polymer); 1.19-5.55 (m, Hm, Hl, Hj, Hk, Hh, CH₂-PEG-Polymer); 2.34 (s, 3H, Ar-CH₃); 6.00 (s, 1H, Ar-H); 6.01-6.63 (d, 1H, J=8.00, Ar-H); 6.64-6.76 (m, 1H, Ar-H) 7.47-7.50 (d, 1H, J=8.72, Ar-H), ¹³C NMR (200 MHz CDCl₃) δ 18.22, 60.12- 72.45, 110.08, 111.86, 113.21, 115.93, 118.99, 126.61, 132.25, 135.33, 150.41, 153.76,155.02, 160.56, 161.88,168.17, Mass Spectrum m/z 472 M⁺.

Scheme 7: Synthesis of PEGylated 7-hydroxy-4-methyl-2H-chromen-2-one (4a)

2.2.8. Synthesis of PEGylated 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid (4b) :

The mono acid derivative of carboxyhydroxy PEG (HO-PEG₂₀₀COOH) (28.0 mmol) was activated with 1:2 molar equivalent of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid (3b) (46.0 mmol) N, N dicyclocarbodiimide (46.0 mmol) was dissolved in dichloromethane. (Scheme 8) The solution was stirred for 24 hrs at room temperature. A syrupy resin obtained was dried under vacuum. The resin was dissolved in 15 mL of acetone. A white precipitate of dicyclohexylurea (DCU) appeared was discarded and filtrate was collected. The final filtrate was evaporated to afford the product. TLC in (ethyl acetate: methanol 8:2) was performed to check the presence of DCU. It showed a negative result. The resin was dried in vacuum for IR, ¹H NMR, ¹³C NMR and Mass characterization. At this stage the resin did not stick anymore to the glass wall. IR spectrum of resin showed the characteristic absorption band PEG ether backbone (1104 cm⁻¹) and absorption bands 1715 cm⁻¹ for the ester, Yield: 90%, density: 1.102cm³, IR (KBr/λmaxcm⁻¹) 3418 (OH-PEG); 2923 (CH₂-PEG), 2876 (-CH₃); 1715 (-C=O, -PEG),1104 (-CH₂-O-CH₂) cm⁻¹, ¹H NMR (400 MHz, CDCl₃/DMSO-d₆) □ (ppm) 1.18 (s,OH-PEG Polymer); 1.63-4.24 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH₂-PEG-Polymer); 2.10 (s, 3Hf, Ar-CH₂); 6.17-6.20 (d, 1Ha, J=9.64, Ar-H), 6.69-6.71 (d, 1Hc, J=7.64, Ar-H); 6.72-6.80 (m, 1Hb, Ar-H), 7.46-7.51 (m, 1Hd, Ar-H),10.49 (s, 1He, Ar -OH), ¹³C NMR (200 MHz CDCl₃) δ 34.52, 60.46- 70.15, 102.51, 112.22, 113.21, 126.67, 149.51, 150.29, 154.99, 155.21, 160.38, 160.56, 161.49, 169.13, 170.84, Mass Spectrum  m/z 502 M⁺

Scheme 8: Synthesis of PEGylated 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid

2.2.9. Synthesis of PEGylated 7-hydroxy-4-methyl-8-nitro-2H-chromen-2-one (4c):

Yield: 92%, density: 1.110cm³, IR (KBr/λmaxcm⁻¹) 3419 (OH-PEG); 2927 (CH₃-PEG), 2874(-CH₃); 1705 (-C=O, -PEG),1104 (-CH₂-O-CH₂) cm⁻¹, ¹H NMR (400 MHz, CDCl₃/DMSO-d₆) □ (ppm) 1.20 (s, Hk, OH-PEG Polymer); 1.64-4.25 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH₂-PEG-Polymer); 2.12 (s, 3Hf, Ar-CH₂); 6.02 (s, 1Hb, Ar-H), 6.07-6.70 (m, 1Hc, Ar -H);  6.71- 6.92 (d, 1Ha, J=8.4, Ar-H). ¹³C NMR (200 MHz CDCl₃) δ 18.42, 60.43-70.15, 110.19, 111.94, 113.00, 115.94, 126.84, 129.63, 133.25, 146.77, 153.33, 159.01, 164.53, 167.00. Mass Spectrum m/z 503 M⁺

2.2.10. Synthesis of PEGylated 7-hydroxy-8-ido-4-methyl-2H-chromen-2-one (4d):

Yield: 94%, density: 1.126cm³, IR (KBr/λmaxcm⁻¹) 3336 (OH-PEG); 2921 (CH₃-PEG), 2875 (-CH₃); 1707 (-C=O, -PEG), 1108 (-CH₂-O-CH₂) cm⁻¹, ¹H NMR (400 MHz, CDCl₃/DMSO-d₆) □ (ppm) 1.01 (s, Hk, OH-PEG Polymer); 1.18-5.54 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH₂-PEG-Polymer); 2.35 (s, 3Hd, Ar-CH₃); 6.00 (s, 1Hb, Ar-H), 6.06-6.68 (m, 1Hc, Ar-H); 6.70-6.92 (d, 1Ha, J=8.8, Ar-H). ¹³C NMR (200 MHz CDCl₃) δ 18.98, 61.16-71.02, 90.00, 112.80, 116.91, 119.23, 131.15, 134.42, 134.98, 151.01, 152.93, 160.44, 165.03, 167.91, Mass Spectrum m/z 584 M⁺
2.2.11. Synthesis of PEGylated 3-bromo-7-hydroxy-4-methyl-2H-chromen-2-one (4e):

Yield: 89%, density: 1.135 cm\(^3\), IR (KBr/\(\lambda_{max}\) cm\(^{-1}\)) 3329 (OH-PEG); 2922 (CH\(_2\)-PEG); 2878 (-CH\(_3\)); 1709 (-C=O, -PEG); 1105 (-CH\(_2\)-O-CH\(_2\)) cm\(^{-1}\), \(^1\)H NMR (400 MHz, CDCl\(_3\)/DMSO-d\(_6\)) \(\delta\) (ppm) 1.01 (s, Hk, OH-PEG Polymer); 1.18-5.54 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH\(_2\)-PEG-Polymer); 2.35 (s, 3Hd, Ar-CH\(_3\)); 6.00 (s, 1Hb, Ar-H), 6.06-6.68 (m, 1Hc, Ar-H); 6.70-6.92 (d, 1Ha, J=8.8, Ar-H), \(^1\)C NMR (200 MHz CDCl\(_3\)) \(\delta\) 18.02, 61.20-73.99, 112.97, 116.34, 117.95, 120.35, 148.22, 152.93, 160.88, 164.05, 167.02, Mass Spectrum \(m/z\) 537 M\(^+\)

<table>
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<th>S.No.</th>
<th>Structures of PEGylated Coumarins</th>
<th>Density (d) in Cm(^3)</th>
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<td>4a</td>
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<td>4e</td>
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2.3. Preparation of Ag Nano particles

In a 1L flask, a solution of 90 mg AgNO\(_3\) in 500 mL of triple distilled H\(_2\)O was brought to boiling with rapid stirring. To this solution was added 10 mL of 1% sodium citrate. The reaction mixture was boiled for 30 min, and then it was diluted up to 420 mL. A series of reduction reactions were characterized by changes in the colour. The reaction mixture of AgNPs were centrifuged twice at 10,000 rpm for 10 min to concentrate the solution. All nanoparticles were collected and stored at room temperature in the dark bottles and were generally used within 1-2 months after preparation. A series of reduction reactions characterized by changes in the colour is depicted in Figure 1(a). All nanoparticles were stored at room temperature in dark bottles and were generally used within 1-2 months after preparation. The TEM images are depicted in Figure 1(b).
2.4. Preparation of Colloidal Gold Nanoparticles:

Gold chloride (4%) solution and 1% sodium citrate solutions were made in deionized H2O (DIH2O). One litre of the 4% gold chloride solution was under reflux during the addition of 80 mL of sodium citrate solution. The addition of sodium citrate solution to the gold chloride initiated a series of reduction reactions characterized by changes in the color of the initial gold chloride solution. After particle synthesis, the solution was cooled to room temperature; the gold nanoparticles were concentrated by centrifugation of the reaction mixture at 16000 rpm for 30 min at room temperature, and then were collected for further characterization. The progress of reaction (color change) of the resulting colloid having small particle size is shown in Figure 2(a) and TEM images in Figure2(b).

2.5. Surface functionalization of silver (4a-e) /gold (5a-e) nanoparticles with pegylated coumarin:

PEGylated-Coumarin prepared as described in the preceding section was added to the aqueous dispersion of gold/silver nanoparticles and the reaction was carried out for conjugation of the fluorescence dye to the gold surface through the alcoholic functionality at room temperature (Scheme 10). Fluorescent conjugated nanoparticles were separated from free thiazole PEG hydroxyl group by centrifugation with several washings.
Attachment of PEGylated coumarin onto the surface of gold/silver nanoparticles was confirmed by the fluorescence that was observed by UV-Visible spectrophotometer.

Scheme 10: Surface functionalization of silver /gold nanoparticles with pegylated coumarins

Table 3: Silver (5a-e) and Gold (6a-e) nanoparticles with substituted PEGylated Coumarin

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Structures of PEGylated Coumarin</th>
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<td>5a</td>
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<td>5c</td>
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2.6. UV-Visible Spectroscopy

The preliminary investigation of formation of the gold and silver nanoparticles was carried out by UV-visible spectroscopic analysis. In case of silver nanoparticles, the absorption was observed at 400 nm; similarly, a medium peak of absorbance at higher wavelength 518 nm was observed for gold nanoparticles. The formation of gold and silver nanoparticles was also supported by TEM analysis. The plots of the absorbance at λmax (nm) vs time (min) of reaction were shown in the sets of Figure 3(a), 3(b) and 3(c) and ratified the rapid formation of nanoparticles in the process. In the Figure 3(d), a signal due to PEGylated coumarin was completely diminished. Moreover, no signal appeared at 518 nm for Au-nanoparticle. A new signal appeared at 518 nm showed a red shift which confirmed the formation of conjugated Au nanoparticles with PEGylated Coumarin. The absorbance at 360 nm confirmed the formation of Ag-nanoparticle linked with PEGylated coumarin Figure (3e). In both the cases, the absorbance recorded in the red shift region, supported surface modification of the nanoparticles loaded with PEGylated Coumarin.

2.7. Transmission electron microscopy analysis (TEM)

The Ag/Au nanoparticles were stable for two months, while those without stabilizer were stable for one week. A well dispersed matrix has been recorded in TEM Figure. The particle diameter was measured from a total of at least 100 randomly selected particles using transmission electron microscopy (TEM, JEOL (JEM 2100) at 120 kV accelerating voltage and 300,000 X magnification, pictures were taken. TEM specimens were prepared by diluting the samples in 1:1 with Mili Q water. Diluted 5µL samples loaded on 200 mesh carbon coated copper grids for 5 minutes. Excess samples were removed by using Whatman filter paper and allowed to dry for 2 hours. Samples were observed using JEOL (JEM 2100) TEM at 120 kV. Images captured at various scale bar 0.2 µm, 0.5 µm and 1 µm etc. The pegylated nanoparticles were generally spherical and of regular size when examined by TEM in previous sections. Most silver and gold nanoparticles were around 4-60 nm or below in diameter.

Figure 3: UV-Vis spectrum of a) gold, (b) silver nanoparticle, (c) PEGylated-Coumarin, (d) silver and (e) gold nanoparticles conjugated with PEGylated coumarin.
few were 100 to 160 nm in diameter. Transmission electron micrographs displayed pegylated coumarin-decorated AgNPs and AuNPs which were visualized by binding to silver and gold nanoparticles of various sizes in nanometres Figure 4(a) and (b).

Figure 4: TEM image of a) gold and b) silver nanoparticles conjugated with PEGylated Coumarins

III. BIOLOGY

In vitro anti-bacterial activity

3.1. General screening procedure:

All the newly synthesized compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains such as Staphylococcus aureus (gram +ve), Escherichia coli (gram -ve), by the cuplet method. The nutrient agar broth were prepared by aseptic inoculation with 0.5 mL of 24 hrs old subcultures of S. aureus, E. coli in separate flasks at 40-50°C and mixing well by gentle shaking. About 25 mL of the contents of the flask were poured and evenly spread in a petri dish (13 cm in diameter) and allowed to set for 2 hrs. Each test compound (20 mg) was dissolved in 2 mL of DMSO, which is used as a sample solution. A concentrated (100, 50 and 25 μg/mL) solutions were prepared by dilution method. The plates were incubated at 37°C for 24 hrs for bacterial strains. The control was similarly maintained with 1mL of DMSO and the zones of inhibition of the bacterial growth were measured in mm using zone reader. PEGylated-coumarins were screened against clinical isolates of gram-positive bacteria Staphylococcus aureus, gram-negative bacteria Escherichia coli The cytotoxicity of all scaffolds was compared with Ampicillin and Doxycycline for antibacterial study and The antibacterial screening was carried out by cup-plate method at different levels of concentration (25, 50, 100 μg/mL) in solvent DMSO.

Table 4: Antibacterial activity of 3, 4 and 5 at 100 /50 /25 μg /mL

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compound No.</th>
<th>Diameter of zone of inhibition in mm</th>
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<tr>
<td></td>
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<td>E. coli 50</td>
</tr>
<tr>
<td>1</td>
<td>4a</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>4b</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>4c</td>
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<tr>
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IV. RESULTS AND DISCUSSION

A new class of surface functionalized PEGylated-coumarins were synthesized. These compounds were used for surface activation on to synthesized gold/silver nanoparticles. Surface plasmon resonance and TEM images of surface functionalized AgNPs and AuNPs PEGylated coumarin suggested that these technologies monitored the noncovalent molecular interactions amongst the metallic nanoparticles and PEGylated coumarin. All PEGylated-coumarins nanoparticles were characterized by CHN, elemental analysis, UV-visible, IR, $^1$HNMR, $^{13}$C NMR,

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<th></th>
<th>11</th>
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<th>13</th>
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<td>30</td>
<td>15</td>
<td>18</td>
<td>29</td>
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Solvent DMSO - - - - - -

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Figure 5: (a) Inhibition in the growth of the E. coli at 25, 50 and 100μg/mL compared with untreated control, standard Ampicillin and Doxycycline

Figure 6: (b) Inhibition in the growth of the Staphylococcus aureus at 25, 50 and 100μg/mL compared with untreated control, standard Ampicillin and Doxycycline
Mass spectral analysis and transmission electron microscopy (TEM). Target analogs 4, 5 and 6 were tested for anti-bacterial activity, against clinical isolates of Gram-positive bacteria Staphylococcus aureus, Gram-negative bacteria E. coli, However E.coli and S. aureus, showed sensitivity to all the scaffolds and had medium to high zone of inhibition. The inhibitory effects of compounds 4, 5 and 6 against these organisms are depicted Table 4. The results were compared with Ampicillin and Doxycycline for antibacterial study and Griseofulvin as standard drugs. (Figures 4-5).

V. CONCLUSION

In this study, two independent approaches that conjugating coumarins to PEG(200) and mounting this passive targeting moieties onto the NPs were adopted to fabricate coumarin-PEG-AuNPs and coumarin-PEG-AgNPs. It was found that, Staphylococcus aureus, gram-negative bacteria and gram-negative bacteria Escherichia coli were highly sensitive to some scaffolds and showed outstanding activity displayed high zones of inhibition. At 25 μg/mL, all synthesized compounds are inactive against Escherichia coli whereas 4a displayed excellent activity. However, it showed moderate sensitivity against all compounds at 50 μg/mL. Amongst all compounds, 4d, 5a, 5d exhibited and exceptionally high activity at 100 μg/mL and rest of the compounds are good enough. In case of Staphylococcus aureus was found to be resistant against all synthesized compounds except 6d at 25 μg/mL tested in the activity. Compounds 5d and 6b showed extraordinary activity and remaining compounds showed good sensitivity at 50 μg/mL. At 100 μg/mL, 4d, 5c, 6d, 6e showed outstanding activity, while rest of the compounds exhibited respectable activity against Staphylococcus aureus.

VI. ACKNOWLEDGEMENTS

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