EVALUATION AND CHARECTERISATION OF HETEROCYCLIC HYBRID MOLECULES WITH AMINO, HYDROXY AND THIOL SPECIFIC SITE PEGYLATED CONJUGATION

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Abstract

We initially synthesized appropriately substituted various heterocycles as Thiazoles, Coumarin and Quinoline, 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 $–NH_2$.Our strategy involves the synthesis of site-specific PEGylation is intensely being utilized to modify macromolecules, biomolecules, and surfaces. Protein PEGylation is able to address the fundamental issues of site-specific conjugation and high-efficiency conjugation. -NH_2 group of Thiazoles, -OH and -COOH group of Coumarin and –SH group of Quinoline Based chalcones on their selective chemical reactivity and PEG reagents provide the best opportunity for efficient and site-specific PEGylation. The resultant series of Pegylated heterocyclic compounds were characterized by various techniques such as FTIR, ^{1}HNMR, ^{13}CNMR, Mass spectral data and elemental analysis.

Keywords: Thizole hybrids, Coumarin hybrid, quinoline base Chalcone derivatives, specific site PEGylation, antibacterial activity.

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1. Introduction

Thiazole is five member ring system containing sulfur and nitrogen heteroatoms at positions-1 and -3, respectively is involved in many of the natural products. For example, the thiazolium ring available in vitamin B1 serves as an electron sink, and its coenzyme structure is important for the decarboxylation of α-keto acids.[1]

Thiazole and its derivatives are very helpful compounds in diverse fields of chemistry including medicine and agriculture.

Coumarins, a class of fused ring heterocycles, occur broadly among natural products and have significance in medicine.[2–4] Numerous natural products with the coumarinic moiety possess stimulating biological and pharmacological properties. Due to their abundance in plants and their low mammalian toxicity, chromone derivatives are present in large amounts in the diet of humans. They are extensively used as, anti-HIV active,[5] antiviral, intisecticidal,[6] anticancer agents, anti histamines[7] and anticoagulants[8,9] herbicides[9] in food additives, perfumes, cosmetics, dyes[10, 11] fluorescent probes and triplet sensitizers.[12] Quinolines play an indispensable role in the oldest medicines used to fight malaria whereas one of the latest quinoline-containing drugs is montelukast (Singulair@), an anti-asthmatic drug. There is a very much increased impetus in this particular area and in organic chemistry in general, towards a more applied approach.Quinoline derivatives were found to have anticancer[13], anti-HIV[14], antibacterial[15], antimalarial[16], anti-inflammatory[17] activities. Indole derivatives also exhibit antimicrobial[18], antibacterial[19], anti-inflammatory[20], antiviral[21], antidiabetic[22], antitumor[23], and anticancer[24] activities. Chalcone[25] (and related compounds "chalconoids") is an aromatic ketone that forms the central core for a diversity of essential biological compounds, which are known collectively as chalcones.There are a number of derivatives of quinolines and chalcones including several natural as well as semi-synthetics molecules. of note, there are some studies suggesting that these two nuclei of quinoline and chalcones can be linked with suitable linkers and that this enhances tremendously the activity of these compounds.

PEGylation methodology has given important theoretical and commercially useful insight, but many more applications can still be exploited. The products already approved by the FDA are clear demonstration of the usefulness of PEGylation in the improvement of therapeutic value of drugs. The most relevant advantages are the prolonged body-residence time, which allows less frequent administrations, the increase in stability towards many enzymes and the reduction of immunogenicity. These advantages of PEGylation allowed this technique to create blockbuster products There are many conjugation strategies and many PEG-based reagents that have been developed to address the central issue of site-specific PEGylation.[26-27] The use of chemical groups that react with primary amines is one of the oldest and most versatile methods for protein conjugation similarly -OH group on one terminous and -COOH group owned from maleimide functionalized at other end of the PEG. In the present study, we executed the synthesis and characterization of hetero-bifunctional PEG with a -OH group on one terminus and a reactive functional group -COOH at other end for conjugation Thiol site-specific PEGylation is intensely being utilized to modify macromolecules, biomolecules, and surfaces.[28-31] Our strategy involves the synthesis of site-specific PEGylation is intensely being utilized to modify macromolecules, biomolecules, and surfaces. Protein PEGylation is able to address the fundamental issues of site-specific conjugation and high-efficiency conjugation through -NH_2 group of Thiazoles, -OH and -COOH group of Coumarin and –SH group of Quinoline Based on their selective chemical reactivity

2. Materials and methods

PEGylated thiazoles, coumarins and Quinolines 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. 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 DMSO-*d*6/CDCl3 on a Bruker Avance II 400 NMRspectrometer. 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.

3. Experimental Work

3.1. General procedure for the preparation of 4-p-toylthiazol-2-amine(2a):

1-1-p-tolylethanone **1a** (30 mmol) and thiourea (20 mmol) were dissolved in rectified spirit. To

the reaction mixture, (10 mmol) bromine or iodine was added. The contents were refluxed on water bath for 12 hours. The reaction mixture was diluted with water and alcohol was distilled off. The solution was filtered. On addition of ammonium hydroxide to the filtrate, thiazole **2a** separated out. It was recrystalized from dilute ethanol. **(Table 1).** The yield was 89%, Melting Point: 136^0C, IR (KBr, λ_{max}/cm^{-1}): 3456 (NH_2); 1637 (C=N); 1491(C-N); 1037-730(C=C-Ar) cm^{-1}, ^{1}H NMR (400 MHz,$CDCl_3$ /DMSO-d_6): δ(ppm) 2.30(s, 3He, CH_3); 3.72 (s, 2Hg, NH_2); 6.78 (s, 1Hf, thiazole-H); 7.12-7.14 (d, 2Hb, c, J=8.04, Ar-H); 7.65-7.67 (d, 2Ha, d, J=8.16, Ar-H), ^{13}C NMR (200MH_Z $CDCl_3$): δ 20.78, 100.29, 125.41, 128.87, 131.96, 136.33, 149.54, 168.20, Mass Spectrum: m/z 190 M^+, CHN calculated: C 63.13, H 5.30, N 14.72, S 16.85, CHN found: C 63.11, H 5.32, N 14.70, S 16.87.

3.2. Preparation of 4-(4-fluorophenyl)thiazol-2-amine (2b):

Yield: 87%, Melting Point: 110^0C, IR (KBr, λ_{max}/cm^{-1}): 3446(NH_2); 1631(C=N); 1409(C-N); 1043-732(C=C-Ar) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 3.89 (s, 2Hf, NH_2); 6.79 (s, 1He, thiazole-H); 7.62-7.65 (d, 2Hb, c, J=8.28, Ar-H); 8.24-7.26 (d, 2Ha, d, J=8.16, Ar-H), ^{13}C NMR (200MH_Z, $CDCl_3$): δ 100.99, 115.00, 128.20, 130.02, 150.00, 161.99, 167.20, Mass Spectrum: m/z 194 M^+, CHN calculated: C 55.65, H 3.63, N 14.42, S 16.51, CHN found: C 55.65, H 3.63, N 14.42, S 16.51.

3.3. Preparation of 4-(4-chlorophenyl)thiazol-2-amine (2c):

Yield: 90%, Melting Point: 168^0C, IR (KBr, λ_{max}/cm^{-1}): 3438(NH_2); 1621(C=N); 1489(C-N): 1033-730(C=C-Ar) cm^{-1}, ^{1}H NMR(400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 3.70 (s, 2Hf, NH_2); 6.61 (s, 1He, thiazole-H); 7.47-7.49 (d, 2Hb, c, J=8.12, Ar-H); 7.81-7.88 (d, 2Ha, d, J=7.28, Ar-H), ^{13}C NMR (200MH_Z, $CDCl_3$): δ100.95, 128.55, 128.99, 131.35, 134.45, 150.05, 169.25, Mass Spectrum: m/z 210M^+, CHN calculated: C 51.31, H 3.35, N 13.30, S 15.22, CHN found: C 51.29, H 3.36, N 13.32, S 15.19

Table 1: Physical characterisation data of substituted thiazole derivatives (2a-c)

Compounds	Mol. Weight	Yield (%)	M.P.(^{0}C)	Mol. Formula
2a	190	89%	136^0C	$C_{10}H_{10}N_2S$
2b	194	87%	110^0C	$C_9H_7FN_2S$
2c	210	90%	168^0C	$C_9H_7ClN_2S$

3.4. Preparation of 7-hydroxy-4-methyl-2H-chromen-2-one(3a):

Resorcinol (0.1 mmol) and ethyl aceto acetate (0.1 mmol) were disslove in H_2SO_4 (75% 20 ml) mixture was stirred well and kept overnight. It was diluted with ice cold water. The solid seprate was crystallise from dilute ethenol to obtained the 7-hydroxy-4-methyl-2H-chromen-2-one **(3a)**. Molecular Formula: $C_{10}H_8O_3$, Yield: 82%, Melting point:186^0C, IR (KBr/$\lambda_{max}$$cm^{-1}$): 3501 (-OH), 1671 (-C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 2.36 (s, 3He, -CH_3), 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), ^{13}C NMR (200 MH_Z $CDCl_3$) **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

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

Anhydrous Citric acid (10gm) was heated with conc. H_2SO_4 (30ml) on a water bath till the evalution of carbon monoxide gas ceased. After cooling, m-Cresol (0.04 mol, 4.32 gm, 4.18 ml) was added followed by conc. H_2SO_4 (10ml). The reaction mixture was kept for 24 hrs at room temperature. It was then poured in to ice cold water and solid seprated was crystallized from dilute ethanol to get 7-methyl coumarin-4-acetic acid **(3b)**. Molecular Formula: $C_{11}H_8O_5$, Yield: 72%, Melting point: 206^0C, IR (KBr/$\lambda_{max}$$cm^{-1}$): 3499 (-OH), 1614 (-C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 2.51 (s, 3He, -CH_2), 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), ^{13}C NMR (200 MH_Z $CDCl_3$) **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

3.6. 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 5^0C 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. H_2SO_4 acid (100 ml) and was then kept in an ice bath. When the temperature inside the flask is below 1^0C, 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 10^0C. 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: $C_{10}H_7NO_5$, Yield: 88%, Melting point: 255^0C, IR (KBr/λ_{max}cm^{-1}): 3507 (-OH), 1671 (-C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 2.50 (s, 3He, -CH_3), 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), ^{13}C NMR (200 MHz $CDCl_3$) **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.

Table 2: Physical characterisation data of substituted coumarin derivatives (3a-3c)

Compound	Mol. Weight	Yield (%)	M.P. (^{0}C)	Mol. Formula
3a	176	82%	186^0C	$C_{10}H_8O_3$
3b	220	72%	206^0C	$C_{11}H_8O_5$
3c	221	88%	255^0C	$C_{10}H_7NO_5$

3.7. Preparation of 2-chloroquinoline-3-carbaldehyde (2):

To the solution of acetanilide (55 mmol, 10 gm) in dry DMF (165 mmol, 12.77 mL) at 0-5^0C, $POCl_3$ (385 mmol, 35.9 mL) was added drop wise and mixture was then stirred at 80-90^0C for 16-19hrs. The mixture was poured on to crushed ice, and solid separated out. The product 2-chloroquinoline-3-carbaldehyde was recrystalized from ethyl acetate and methanol.

3.8. Preparation of 3-(2-chloroquinoline-3-yl)-1-(4-flurophenyl)prop-2-en-1-one (4a):

A mixture of 2-chloroquinoline-3-carbaldehyde (3.39 mmol, 0.7g) and 4-methyl acetophenone (2.3 mmol, 0.311 mL) in 40% ethanolic NaOH was stirred vigorously for 2hr and was kept overnight at room temperature**.** The reaction mixture was poured onto crushed ice and acidified with 1:1 HCl. The solid 3-(2-chloroquinoline-3-yl)-1-p-tolylprop-2-en-1-one was isolated. Yield: 87%, Melting point 175^0C, IR (KBr/λ_{max}cm^{-1}) 3053 (CH=CH), 1682 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6), δ(ppm) 6.24-6.26 (d, 1Hf, J=8.12, -CH), 6.54-6.58 (m, 4H, Ha, Hb, Hc, Hd), 6.83-7.85 (d, 1Hg, J=8.16, -CH), 7.05 (s, 1H, He), 7.09-7.14 (m, 2H, Hh, Hi, Ar-H), 7.15-7.21 (m, 2H, Hk, Hj, Ar-H),^{13}C NMR(200 MHCDCl_3) **δ** 115.23, 125.12, 126.14, 132.21, 133.17, 134.31, 145.01, 146.26, 149.31, 167.90, 190.91, Mass Spectrum: m/z 311 M^+.

3.8. Preparation of 3-(2-chloroquinoline-3yl)-1-*p*-tolyl-prop-2en-1-one (4b) :

Yield: 73%, Melting point 204^0C, IR (KBr/λ_{max}cm^{-1}) 3066 (CH=CH), 1657 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6), δ(ppm) 2.23 (s, 1H, -CH_3), 6.38-6.40 (d, 1Hf, J=8.12, -CH), 6.53-6.67 (m, 4H, Ha, Hb, Hc, Hd), 6.68-6.70 (d, 1Hg, J=8.06, -CH), 6.98 (s, 1H, He), 7.27-7.39 (m, 2H, Hh, Hi, Ar-H), 7.43-7.66 (m, 2H, Hk, Hj, Ar-H), ^{13}C NMR(200MHz, $CDCl_3$) **δ** 21.02, 126.25, 127.14, 130.13, 130.27, 134.37, 135.56, 144.28, 145.02, 146.09, 149.01, 189.82, , Mass Spectrum: m/z 307 M^+.

3.9. Preparation of 3-(2-chloroquinoline-3-yl) 1-(4-nitrophyenyl)prop-2en-1-one (4c)

Yield: 75%, Melting point 180^0C, IR (KBr/λ_{max}cm^{-1}) 3062 (CH=CH), 1650 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6), δ(ppm) 6.74-6.76 (d, 1Hf, J=8.12, -CH), 6.80-7.12 (m, 4H, Ha, Hb, Hc, Hd), 7.33-7.36 (d, 1Hg, J=8.16, -CH), 7.40 (s, 1H, He), 7.45-7.75 (m, 2H, Hh, Hi, Ar-H), 7.82-7.93 (m, 2H, Hk, Hj, Ar-H), ^{13}C NMR(200MHz, $CDCl_3$) **δ** 124.03, 126.26, 127.44, 130.17, 135.27, 144.34, 145.16, 146.08, 149.42, 153.09, 189.22, Mass Spectrum: m/z 338 M^+.

Table 3: Physical characterization data of substituted quinoline derivatives (4a-e)

Compound	Mol. Weight	Yield (%)	M.P. (^{0}C)	Mol. Formula
4a	309	82%	197^{0}C	$C_{18}H_{11}FNOCl$
4b	305	71%	204^{0}C	$C_{19}H_{14}NOCl$
4c	336	73%	180^{0}C	$C_{18}H_{11}N_2O_3Cl$

3.10. Preparation of 1-(4-fluorophenyl)3-(2-mercaptoquinoline-3-yl) prop-2-en-1-one (5a):
A mixture of 2-chloroquinoline-3-carbaldehyde (3.39 mmol, 0.7 g) and 4-fluro-acetophenone (2.3 mmol, 0.311 mL) in 40% ethanolic NaOH was stirred vigorously for 2hr and was kept overnight at room temperature**.** The reaction mixture was poured onto crushed ice and acidified with 1:1 HCl. The solid 1-(4-fluorophenyl)3-(2-mercaptoquinoline-3-yl) prop-2-en-1-one was isolated. **Scheme 1**., Yield: 87%, Melting point 197^{0}C, IR (KBr/λ_{max}cm^{-1}) 3061 (CH=CH), 1655 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6) δ(ppm) 3.45 (s, 1H, -SH), 7.59-7.83 (m, 2H, Hh, Hi, Ar-H), 7.84-7.94 (m, 4H, Ha, Hb, Hc, Hd), 8.04-8.05 (d, 1Hg, J=3.76, -CH), 8.06-8.18 (m, 2H, Hh, Hi, Ar-H), 8.78 (s, 1H, He, Ar-H), 9.40-9.42 (d, 1H, Hf, J= 3.76, -CH) ^{13}C NMR(200 MHz, $CDCl_3$) **δ** 116.18, 122.10, 126.24, 127.94, 128.10, 129.53, 131.26, 133.96, 137.96, 145.80, 146.88, 168.23, 177.16, 189.50, Mass Spectrum m/z 309 M$^+$, CHN calculated C 69.88, H 3.91, N 4.53, S 10.37, CHN found C 69.84, H 3.87, N 4.49, S 10.33.

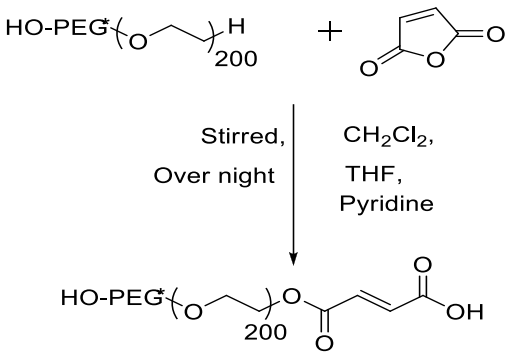
3.11. Preparation of 3-(2-mercaptoquinolin-3-yl)-1-(p-tolyl)prop-2-en-1-one (5c):
Molecular Formula: Yield: 73%, Melting point 140^{0}C, IR (KBr/λ_{max} cm^{-1}) 3066 (CH=CH), 1657 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6) δ(ppm) 2.38 (s, 3H, -CH_3), 3.52 (s, 1Hf, -SH), 7.28-7.31 (d, 1H, J=8.13, -CH), 7.43-7.62 (m, 4H, Ha, Hb, Hc, Hd), 7.65-7.67 (d, 1H, J=8.16, -CHg), 7.12 (s, 1H,Hd), 8.02-8.21 (m, 2H, Ar-H), 8.22-8.35 (m, 2H, Ar-H), ^{13}C NMR (200 MH_Z, $CDCl_3$), **δ** 21.50, 122.79, 126.00, 127.20, 127.32, 128.81, 128.99, 129.79, 129.82, 129.86, 129.93, 129.99, 134.86, 135.34, 144.20, 145.95, 146.83, 177.53, 189.33, Mass Spectrum: m/z 305 M$^+$, CHN calculated C 74.72, H 4.95, N 4.59, S 10.50 , CHN found C 74.68, H 4.90, N 4.55, S 10.45.

3.12. Preparation of 3-(2-mercaptoquinolin-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one (5d):
Molecular Formula: $C_{18}H_{12}N_2O_3S$, Yield: 75%, Melting point 150^{0}C, IR (KBr/λ_{max} cm^{-1}), 3062(CH=CH), 1650 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6), δ(ppm) 3.47 (s, 1H, -SH), 7.17-7.19 (d, 1H, J=8.02, -CH), 7.49-7.96 (m, 4H, Ha, Hb, Hc, Hd), 7.63 (s, 1H, -Ar-H), 7.85 (s, 1H, Hd), 8.37-8.39 (d, 1H, Ar-H), 8.15-8.17 (m, 2H, Ar-H), ^{13}CNMR (200MH_Z, $CDCl_3$) **δ** 122.21, 122.32, 126.34, 127.83, 127.99, 128.86, 128.99, 130.01, 135.39, 144.25, 145.88, 146.98, 153.33, 177.12, 189.78, Mass Spectrum m/z 336 M$^+$, CHN calculated C 65.37, H 3.50, N 8.32, S 9.13, CHN found C 64.32, H 3.54, N 8.08, S 9.07.

Table 4: Physical characterisation data of substituted quinoline derivatives (5a-c)

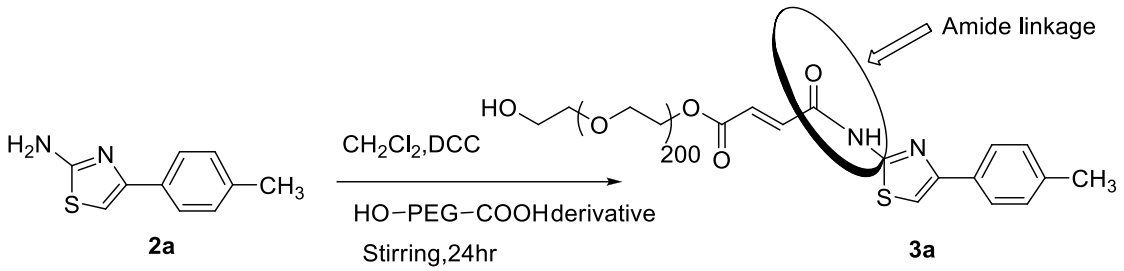
Compound	Mol. Weight	Yield (%)	M.P. (^{0}C)	Mol. Formula
5a	309	87%	197^{0}C	$C_{18}H_{12}FNOCl$
5b	305	73%	140^{0}C	$C_{19}H_{15}NOCl$
5c	336	75%	150^{0}C	$C_{18}H_{12}N_2O_3Cl$

3.13. Synthesis of Hydroxycarboxy poly ethylene glycol (HO-PEG$_{200}$COOH):
Polyethylene glycol (28.0 mmol, 5ml 200 gm/mol) was disslove in 20 ml of dry CH_2Cl_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)$_{200}$ was used without purification. **Scheme 8**



Scheme 8: Synthesis of Hydroxycarboxy poly ethylene glycol (HO-PEG$_{200}$COOH)

3.14. Synthesis of N-Terminal substituted PEGylated-4-p-toylthiazol-2-amine (3a):
The mono acid derivative of hydroxycarboxy PEG (HO-PEG_{200}COOH) (28.0 mmol) was activated with 1:2 molar equivalent of 4-p-tolylthiazol amine (46.0 mmol) and N, N dicyclocarbidiimide (46.0 mmol) was dissolved in dichloromethane. The solution was stirred for 24 hrs at room temperature. A syrupy resin was dried under vacuum. A syrupy resin was dissolved in 15 ml of acetone. A white precipitate of dicyclohexylurea (DCU) that appeared was discarded and filtrate was collected. The final filtrate was evaporated to afford the product. TLC (methanol: ethyl acetate 7:3) was performed to check the presence of DCU. A small portion of the filtrate was dissolved in alcohol/water. To this, a solution of Na_2HCO_3 was added. No effervescence was observed, indicated that complete amino group capping was effectively done. A oven dried resin was used in further analysis, UV-visible, IR, ^{1}HNMR,^{13}CNMR and mass characterisation. At this stage the resin did not stick anymore to the glass wall. IR spectrum of resin showed the characteristic absorption band for PEG ether backbone at (1101 cm-1) and 1621 cm^{-1} for the amide bond. Syrupy liquid, Yield: 93%, density: 1.137cm^3, IR (KBr, λ_{max}/cm^{-1}): 3391 (OH, -NH); 2927(CH_2-PEG), 2871(-CH_3); 1621(-C=O, -PEG), 1101(-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ (ppm) 1.10 (s, Hn, OH-PEG Polymer); 1.60-5.90 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH_2-PEG-Polymer); 2.35 (s, 3He, Ar-CH_3); 3.59 (s, 1Hg, -NH); 6.58 (s, 1Hf, thiazole-H); 7.14-7.16 (d, 2H, J=8.12, Hb, Hc, Ar-H) 7.61-7.63(d, 2H, J=8.02, Ha, Hd, Ar-H), ^{13}C NMR (200MH_Z, $CDCl_3$): δ 20.85, 60.45-72.51, 100.66, 125.65, 129.14, 129.27, 132.43, 136.51, 150.14, 168.37, 175.28, Mass Spectrum: m/z 472 M^+, Molecular Formula: PEG-$C_{10}H_9N_2S$



Scheme 9: Synthesis of N-Terminal substituted PEGylated 4-p-toylthiazol-2-amine (3a)

3.15. Synthesis of N-Terminal substituted PEGylated-4-(4-fluorophenyl)thiazol-2-amine (3b): Yield: 91%, density: 1.022 cm^3, IR (KBr, λ_{max}/cm^{-1}): 3339(OH, -NH); 2932 (-CH_2); 1699(-C=O, -PEG) cm^{-1}, 1137(-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 1.61 (s, Hm, OH-PEG Polymer); 1.94-5.02 (m, Hl, Hk, Hj, Hi, Hh, Hg, CH_2-PEG-Polymer, -NH merged at polymer PEG); 7.26 (s, 1He, thiazole-H); 7.78-7.80 (d, 2H, J=8.02, Hb, Hc, Ar-H); 8.32-8.34 (d, 2H, Ha, Hd, J=8.02, Ar-H), ^{13}C NMR (200MH_Z, $CDCl_3$): δ 59.00, 72.63, 105.33, 116.52, 127.84, 129.00, 130.98, 135.92, 151.00, 162.87, 164.00, 166.89, Mass Spectrum: m/z 476 M^+, Molecular Formula: PEG-$C_9H_6FN_2S$.

3.16. Synthesis of N-Terminal substituted PEGylated-4(4-chlorophenyl) thiazol-2-amine (3c): Yield: 90%, density: 1.134, IR (KBr, λ_{max}/cm^{-1}): 3338 (OH, -NH); 2929 (-CH_2); 1698 (-C=O, -PEG), 1091 (-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6): δ(ppm) 1.03 (s, Hm, OH-PEG Polymer); 1.60-4.70 (m, Hl, Hk, Hj, Hi, Hh, Hg, CH_2-PEG-Polymer, -NH merged at polymer PEG); 6.75 (s, 1He, thiazole-H); 7.71-7.73 (d, 2H, J=8.02, Hb, Hc, Ar-H); 7.74-7.76 (d, 2H, J=8.02, Ha, Hd, Ar-H), ^{13} C NMR (200MH_Z $CDCl_3$): δ 60.24-79.08, 101.92, 128.22, 131.58, 133.62, 148.60, 164.67, 168.29., Mass Spectrum: m/z 492 M^+, Molecular Formula: PEG-$C_9H_6ClN_2S$

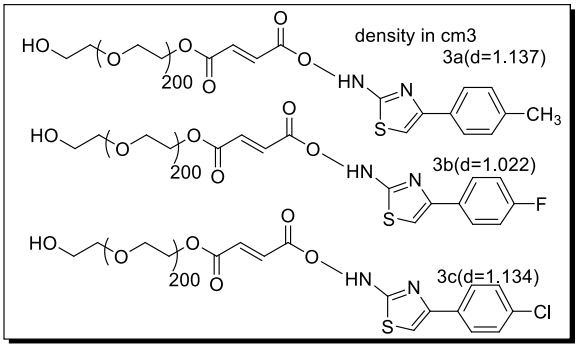
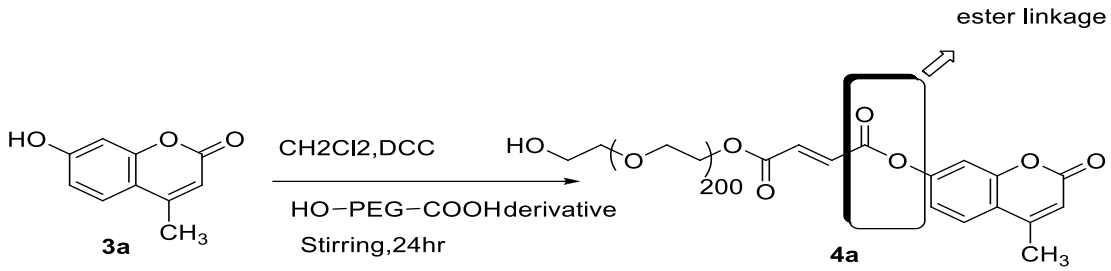


Figure 1: Structures and physical constant of PEGylated thiazoles with amine conjugation (3a-c)

3.17. Synthesis of Oxy-Terminal PEGylated 7-hydroxy-4-methyl-2H-chromen-2-one(4a):
Scheme 10 Yield : 93%, density: 1.043cm^3, IR (KBr/$\lambda_{max}cm^{-1}$) 3334(OH-PEG); 2928 (CH_2-PEG), 2872(-CH3); 1702(-C=O, -PEG), 1103(-CH_2-O-

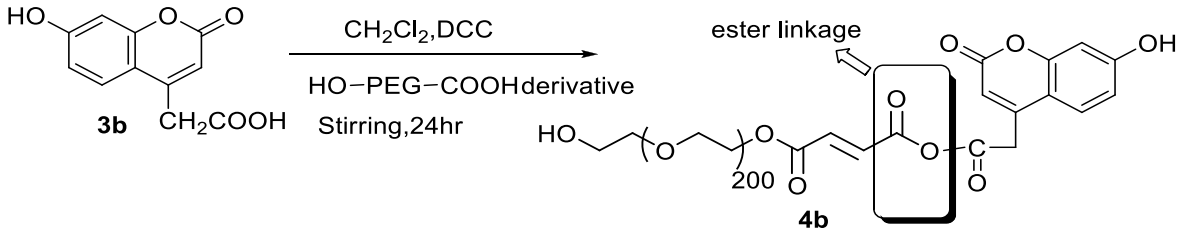
CH_2) cm^{-1}, ^1H NMR (400 MHz,$CDCl_3$ /DMSO-d_6) □ (ppm) 1.02 (m, OH-PEG Polymer); 1.19-5.55 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH_2-PEG-Polymer); 2.34 (s, 3H, Ar-CH_3); 6.00 (s, 1H, Ar-H), 6.01-6.63 (d, 1H, J=8.00, Ar-H); 6.64-6.76 (m, 1H, Ar-H) 7.47-7.50 (d, 1H, J=8.72, Ar-H), ^{13}C NMR (200 MH_Z $CDCl_3$) **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 10: Synthesis of PEGylated 7-hydroxy-4-methyl-2H-chromen-2-one (4a)

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

Scheme 11 Yield: 90%, density: 1.102cm^3, IR (KBr/$\lambda_{max}cm^{-1}$) 3418 (OH-PEG); 2923 (CH_2-PEG), 2876 (-CH_3); 1715 (-C=O, -PEG),1104 (-CH_2-O-CH_2) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$ /DMSO-d_6) □ (ppm) 1.18 (s,OH_l-PEG Polymer); 1.63-4.24 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH_2-PEG-Polymer); 2.10 (s, 3Hf, Ar-CH_2); 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), ^{13}C NMR (200 MH_Z, $CDCl_3$) **δ** 34.52, 60.46-72.51, 102.51, 112.22, 113.21, 126.67, 149.51, 150.29, 154.99, 155.21, 155.93, 160.38, 160.56, 161.49, 169.13, 169.26, 170.84, Mass Spectrum m/z 502 M^+



Scheme 11: Synthesis of PEGylated 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid

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

Yield: 92%, density: 1.110cm^3, IR (KBr/$\lambda_{max}cm^{-1}$) 3419 (OH-PEG); 2927 (CH_2-PEG), 2874(-CH_3); 1705 (-C=O, -PEG), 1101 (-CH_2-O-CH_2) cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$ /DMSO-d_6)□□ (ppm) 1.19 (s, Hk, OH-PEG Polymer); 1.64-4.25 (m, Hm, Hl, Hk, Hj, Hi, Hh, CH_2-PEG-Polymer); 2.12 (s, 3Hd, Ar-CH_3); 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). ^{13}C NMR (200 MH_Z $CDCl_3$) 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^+

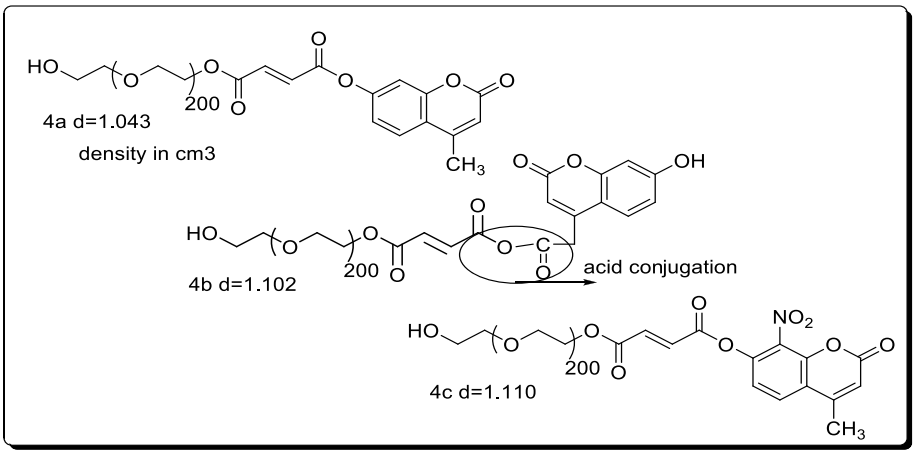
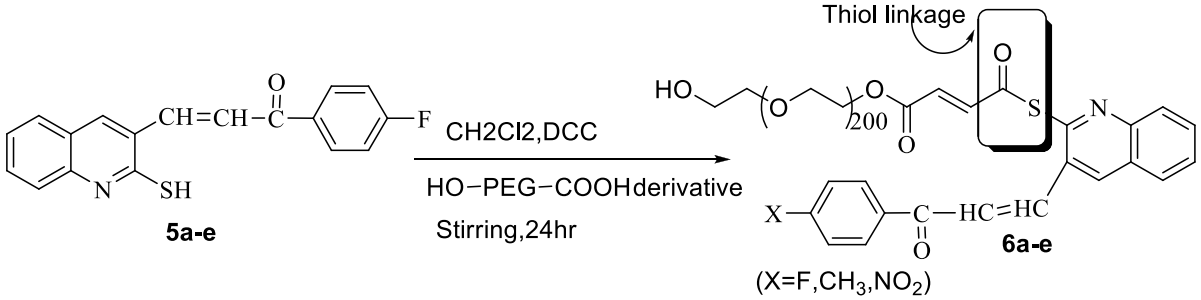


Figure 2: Structures of PEGylated Coumarin derivatives hydroxy and acid conjugation4(a-c)

3.20. Synthesis of substituted PEGylated-1-(4-fluorophenyl)-3-(2-mercaptoquinoline-3-yl)prop-2-en-1-one (6a):

Scheme 12 Yield: 93%, IR (KBr/λ_{max}cm^{-1}) 3410 (-OH-PEG), 2925 (CH_2-PEG), 1646(-C=O, -PEG), 1100 (-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6) □(ppm) 1.62 (s, 1H, OH-PEG Polymer); 1.94-4.03 (m, CH_2-PEG-Polymer), 7.44-7.46 (d, 1H, J=7.96, -CH), 7.12 (s, 1H, He), 7.13-7.16 (m, 4H, Ar-H), 7.17-7.26 (m, 2H, Hb, Hc), 8.09-8.10 (d, 1H, J=2.28, -CH), 8.52 (s, 1Ha, Ar-H), 8.53 (s, 1Hd, Ar-H), ^{13}C NMR (200 MHz $CDCl_3$) □□60.25, 63.43, 69.76, 69.81, 69.84, 72.34, 116.18, 122.08, 124.51, 127.78, 127.82, 128.86, 128.98, 129.99, 131.88,133.46, 135.86, 139.99, 147.37, 149.91, 166.52, 168.78, 177.28, 187.88, 189.54, Mass Spectrum m/z 591 M$^+$, Molecular Formula: PEG-$C_{18}H_{12}FNOS$.



Scheme 12: Synthesis of PEGylated-1-(4-fluorophenyl)3-(2-mercaptoquinoline-3-yl)prop-2-en-1-one

3.21. Synthesis of PEGylated-3-(2-mercaptoquinolin-3-yl)-1-(p-tolyl)prop-2-en-1-one (6c):

Yield: 94%, IR (KBr/λ_{max}cm^{-1}) 3415 (-OH-PEG), 2935 (CH_2-PEG), 1636 (-C=O, -PEG),1103 (-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6), □(ppm) 2.73 (s, 1H, -CH3), 1.06 (s, 1H, OH-PEG Polymer); 1.20-4.62 (m, CH_2-PEG-Polymer), 7.10-7.21 (d, 1H, J=7.97, -CH), 7.15-7.16 (m, 2H, He), 7.17-7.28 (m, 2H, Ar-H), 7.29-7.53 (m, 4H, Ar-H), 7.54-7.73 (d, 1H, J=7.9, -CH), 8.22 (s, 1Ha, Ar-H), ^{13}C NMR (200 MHz $CDCl_3$) **δ** 60.25, 63.43, 69.76, 69.81, 69.84, 72.34, 116.18, 122.08, 124.51, 127.78, 127.82, 128.86, 128.98, 129.99, 131.88,133.46, 135.86, 139.99, 147.37, 149.91, 166.52, 168.78, 177.28, 187.88, 189.54, Mass Spectrum m/z 587 M$^+$

3.22 Synthesis of PEGylated-3-(2-mercaptoquinolin-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one (6d):

Yield: 95%, IR (KBr/λ_{max}cm^{-1}) 3412 (-OH-PEG), 2923 (CH_2-PEG), 1648 (-C=O, -PEG), 1106 (-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6) □(ppm) 1.07 (s, 1H, OH-PEG Polymer); 1.21-4.60 (m, CH_2-PEG-Polymer), 7.09-7.11 (d, 1H, J=7.96, -CH), 7.13-7.14 (m, 2H, He), 7.15-7.25 (m, 2H, Ar-H), 7.26-7.52 (m, 4H, Hb, Hc), 7.53-7.72 (d, 1H, J=7.6, -CH), 8.20 (s, 1Ha, Ar-H), **δ** 60.80, 64.14, 70.30, 70.35, 70.37, 70.40, 72.90, 123.72, 123.85, 125.92, 127.82, 129.35, 129.40, 130.48, 130.54, 132.26, 133.88, 139.95, 144.61, 146.40, 148.34, 149.98, 156.87, 169.22, 189.54, Mass Spectrum m/z 618 M$^+$

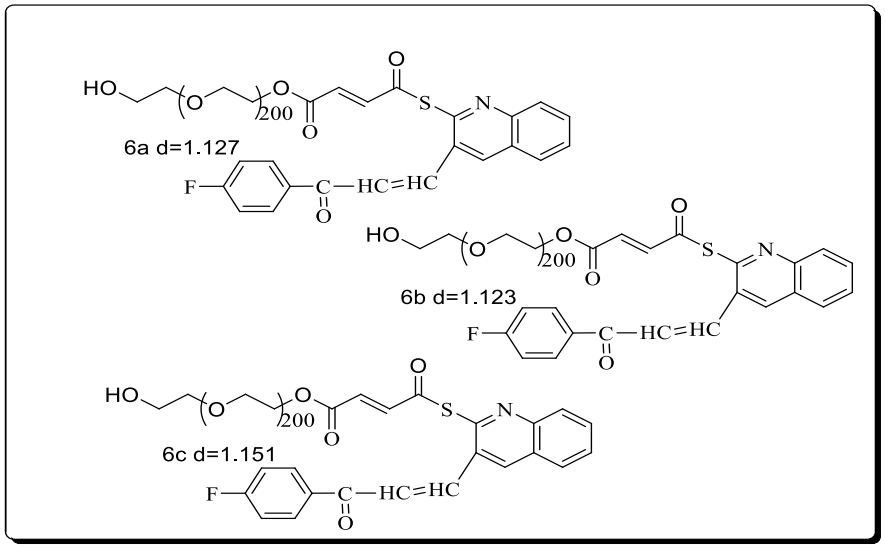


Figure 3: Physical data of PEGylated quinolines (6a-c)

4. Results and discussion

We have synthesized various differently substituted thiazoles, Coumarin, quinoline and its derivatization of privileged structures. We have generated important pathways for synthons deemed promising for the synthesis of other important derivatives of above hybrid molecules. Introduction of substituents like hydroxyl, halogen, nitro, methoxy groups is facile. Efficient syntheses of 9 appropriately substituted PEGylated-thaizoles**Fig.1(3a-3c)**, PEGylated-coumarin **Fig.2(4a-4c)** and PEGylated-quinoline based chalcone **Fig.3(6a-6c)** has been achieved, which leads site-specific conjugation and high-efficiency conjugation i.e. -NH_2 group of Thiazoles with PEG (amine conjugation), -OH and –COOH group of Coumarin(hydroxyl or acid conjugation and –SH group of Quinoline Based chalcone (thiol conjugation) on their selective chemical reactivity and PEG reagents provide the best opportunity for efficient and site-specific PEGylation. .All PEGylated-hybrid molecules were characterized by CHN, elemental analysis, IR, ^{1}HNMR, ^{13}C NMR, Mass spectral analysis.

4. Conclusion

In this study, We prepared high value Thiazole, coumarine and quinoline derivatives heterocyclic hybrid molecules, involves the conjugation of site-specific PEGylation and high-efficiency conjugation. via -NH_2 group of Thiazoles, -OH and -COOH group of Coumarin and –SH group of Quinoline Based chalcones on their selective chemical reactivity and PEG reagents provide the best opportunity for efficient and site-specific PEGylation.

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