SYNTHESIS AND EVALUATION OF THIOL SPECIFIC SITE PEGYLATED CHALCONE QUINOLINE BASED MOLECULAR HYBRIDS AS POTENTIAL ANTI-BACTRIAL AGENTS

Sonali S. Rahate[1*], Anjali M. Rahatgaonkar[2]

Abstract

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. Thiol 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. Based on their selective chemical reactivity, reactive thiol and PEG reagents provide the best opportunity for efficient and site-specific PEGylation. However, to use a thiol reactive PEG reagent, it is necessary to recombinantly engineer a new moiety for further evalution. To prepare the new molecular hybrids, the quinoline nucleus, one of the privileged scaffolds, was coupled with various chalcone derivatives accommodate thiol reactive site via an appropriate PEG linker to produce a total of five PEGylated molecular hybrids The synthesized compounds displayed novel antibacterial activity.

Keywords: quinoline hybrids, Chalcone derivatives, Thiol specific site PEGylation, antibacterial activity.

[1*,2]Department of Chemistry, Institute of Science, Civil Lines, Nagpur 440001

[*]**Corresponding Author:** Sonali S. Rahate
Department of Chemistry, Institute of Science, Civil Lines, Nagpur 440001, sonalisamarth666@gmail.com

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1. Introduction

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. Interest is especially focused on the importance of the quinoline nucleus in natural and physiologically active structures and molecules of actual or potential drug used. Emphasis has increased on natural product synthesis, and bio- and biomimetic chemistry, and the quinoline structure has also figured in the formulation of combinatorial libraries. The accumulation of such libraries represent a quantum jump in synthetic methodology, encompassing the making and screening of thousands of compounds with systematic structural variations. Many quinolines display interesting physiological activities and have found attractive applications as pharmaceuticals and agrochemicals as well as being general synthetic building blocks. Quinoline derivatives were found to have anticancer[1], anti-HIV[2], antibacterial[3], antimalarial[4], anti-inflammatory[5] activities. Indole derivatives also exhibit antimicrobial[6], antibacterial[7], anti-inflammatory[8], antiviral[9], antidiabetic[10], antitumor[11], and anticancer[12] activities.

Chalcone[13] (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. Chalcones are naturally occurring compounds established in several plant species like *Angelica, Glycyrrhiza, Humulus* and *Scutellaria*, which are broadly used as traditional folk remedies. Chalcones are intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities. These are abundant in edible plants and are considered to be precursors of flavonoids and isoflavonoids.[14]

Thiol site-specific PEGylation is intensely being utilized to modify macromolecules, biomolecules, and surfaces.[15-18] Protein PEGylation is able to address the fundamental issues of site-specific conjugation and high-efficiency conjugation. There are many conjugation strategies and many PEG-based reagents that have been developed to address the central issue of site-specific PEGylation.[21-22] Based on their selective chemical reactivity, thiol reactive PEG reagents provide the best opportunity for efficient and site-specific PEGylation.

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.[23] 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).[24] The synthesized various chalcone derivatives accommodate thiol reactive site via an appropriate PEG linker to produce a total of five PEGylated molecular hybrids 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.

2. Experimental Work

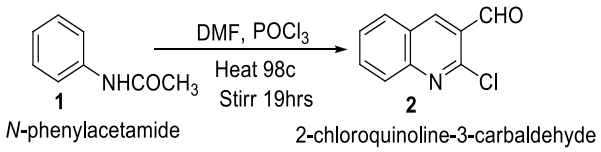
2.1Material and methods

Chloroauric acid ($HAuCl_4.4H_2O$) was obtained from Acros Organics (New Jersey, USA), Silver nitrate ($AgNO_3$), $NaBH_4$, 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 quinoline based chalcones 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 DMSO-*d*6/CDCl3 on a Bruker Avance II 400 NMR spectrometer.

2.2. General Procedure

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

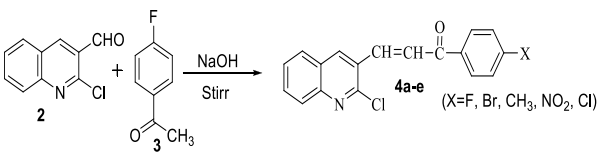
To the solution of acetanilide (55 mmol, 10 gm) in dry DMF (165 mmol, 12.77 mL) at 0-5^{0}C, $POCl_3$ (385 mmol, 35.9 mL) was added drop wise and mixture was then stirred at 80-90^{0}C for 16-19hrs. The mixture was poured on to crushed ice, and solid separated out. The product 2-chloroquinoline-3-carbaldehyde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



Scheme 1: Synthesis of 2-chloroquinoline-3--3-carbaldehyde

2.2.2. 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. **Scheme 3** Yield: 87%, Melting point 175^{0}C, 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$^+$.



Scheme 2: Synthesis of 3-(2-chloroquinoline-3-yl)-1-(4-flurophenyl) prop-2-en-1-one (4a-e)

2.2.3. Preparation of 1-(4-bromophyenyl)-3-(2-chloroquinoline-3yl)prop-2en-1-one (4b) :

Yield: 88%, Melting point 223^{0}C, IR (KBr/λ_{max}cm^{-1}) 3059 (CH=CH), 1654 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d_6), δ(ppm) 6.33-6.35(d, 1Hf, J=8.12, -CH), 6.40-6.60 (m, 4H, Ha, Hb, Hc, Hd), 6.73-6.75(d, 1Hg, J=8.16, -CH), 7.32 (s, 1H, He), 7.35-7.42 (m, 2H, Hh, Hi, Ar-H), 7.45-7.61 (m, 2H, Hk, Hj, Ar-H), ^{13}C NMR(200MHz, $CDCl_3$) **δ** 126.22, 127.18, 128.14, 128.91, 130.27, 132.31, 135.11, 136.27, 145.31, 146.90, 149.61, 189.12, Mass Spectrum: m/z 372 M$^+$.

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

Yield: 73%, Melting point 204^{0}C, 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$^+$.

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

Yield: 75%, Melting point 180^{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) 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$^+$.

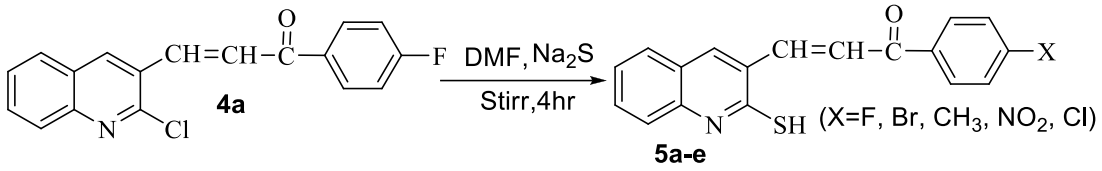
2.2.6. Preparation of 1-(4-chlorophyenyl)-3-(2-chloroquinoline-3yl)prop-2en-1-one (4e)

Yield: 82%, Melting point 209^{0}C, IR (KBr/λ_{max}cm^{-1}) 3069 (CH=CH), 1651 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6), δ(ppm) 6.88-6.90 (d, 1Hf, J=8.11, -CH), 7.01-7.25(m, 4H, Ha, Hb, Hc, Hd), 7.31-7.33 (d, 1Hg, J=8.16, -CH), 7.38 (s, 1H, He), 7.42-7.53 (m, 2H, Hh, Hi, Ar-H), 7.59-7.67 (m, 2H, Hk, Hj, Ar-H), ^{13}C NMR(200MHz, $CDCl_3$) **δ** 126.13, 127.16, 129.42, 130.18, 135.23, 140.31, 145.26, 146.03, 149.22, 189.12 , Mass Spectrum: m/z 327 M$^+$.

Table 1: Physical characterization data of substituted quinoline derivatives (4a-e)

S. No.	Mol. Weight	Yield (%)	M. P. (^{0}C)	Mol. Formula
4a	309	82%	197	$C_{18}H_{11}FNOCl$
4b	370	80%	223	$C_{18}H_{11}BrNOCl$
4c	305	71%	204	$C_{19}H_{14}NOCl$
4d	336	73%	180	$C_{18}H_{11}N_2O_3Cl$
4e	325	85%	209	$C_{18}H_{11}INOCl$

2.3.1. 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 4**., 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.



Scheme 3: Synthesis of 1-(4-fluorophenyl)3-(2-mercaptoquinoline-3-yl)prop-2-en-1-one(5a-e)

2.3.2. Preparation of 1-(4-bromophenyl)-3-(2-mercaptoquinolin-3-yl)prop-2-en-1-one (5b):
Yield: 88%, Melting point 210^{0}C, IR (KBr/λ_{max}cm^{-1}) ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6) δ(ppm) 3.47 (s, 1H, -SH), 7.50-7.60 (m, 2H, Ar-H), 7.61-7.73 (m, 4H, Ha, Hb, Hc, Hd), 7.79-7.83 (m, 2H, Ar-H), 7.83-7.90 (d, 1H, J=2.4 ,-CH), 7.90-8.11 (d, 1H, J=8.01, -CH), 8.35 (s, 1H, Ar-H). ^{13}C NMR (200 MH_Z $CDCl_3$) **δ** 122.11, 127.04, 127.73, 127.89, 128.16, 128.88, 129.82, 132.19, 137.71, 138.40, 138.59, 140.78, 145.38, 177.42, 189.22, Mass Spectrum m/z 370 M$^+$, CHN calculated C 58.39, H 3.27, N 3.78, S 8.66, CHN found C 58.35, H 3.23, N 3.74, S 8.63.

2.3.3. 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.

2.3.4. 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.

2.3.5. Preparation of 1-(4-chlorophenyl)-3-(2-mercaptoquinolin-3-yl)prop-2-en-1-one (5e):
Molecular Formula: $C_{18}H_{12}ClNOS$, Yield: 82%, Melting point 163^{0}C, IR (KBr/λ_{max}cm^{-1}) 3069 (CH=CH), 1651 (C=O), ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6) δ(ppm) 3.29 (s, 1H, -SH), 7.34-736 (d, 1H, J=8.10, -CH), 7.42-7.61 (m, 4H, Ha, Hb, Hc, Hd), 7.61-7.64 (d, 1H, J=8.00, -CH),

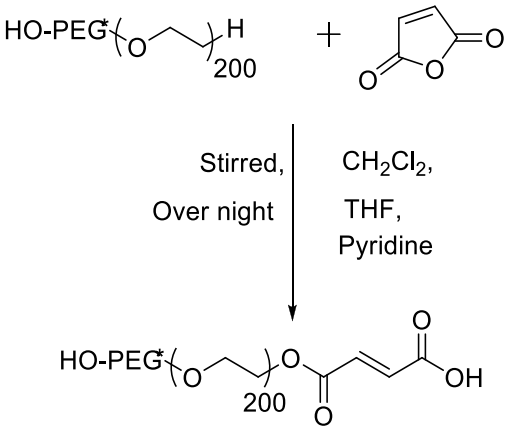
7.82 (s, 1H,Hd), 8.09-8.14 (m, 2H, Ar-H), 7.91-8.23 (m, 2H, Ar-H), ^{13}C NMR (200 MH_Z $CDCl_3$) **δ** 123.01, 126.60, 127.78, 127.89, 128.47, 128.55, 129.99, 130.02, 135.84, 137.09, 138.42, 147.81, 148.13, 177.92, 189.85, Mass Spectrum m/z 417 M^+, CHN calculated C 51.81, H 2.90, N 3.36, S 7.68 CHN found C 51.78, H 2.88, N 3.31, S 7.62.

Table 2: Physical characterisation data of substituted quinoline derivatives (5a-e)

S. No.	Mol. Weight	Yield (%)	M. P. (^{0}C)	Mol. Formula
5a	309	87%	197	$C_{18}H_{12}FNOS$
5b	370	85%	210	$C_{18}H_{12}BrNOS$
5c	305	73%	140	$C_{19}H_{15}NOS$
5d	336	75%	150	$C_{18}H_{12}N_2O_3S$
5e	325	82%	163	$C_{18}H_{12}ClNOS$

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

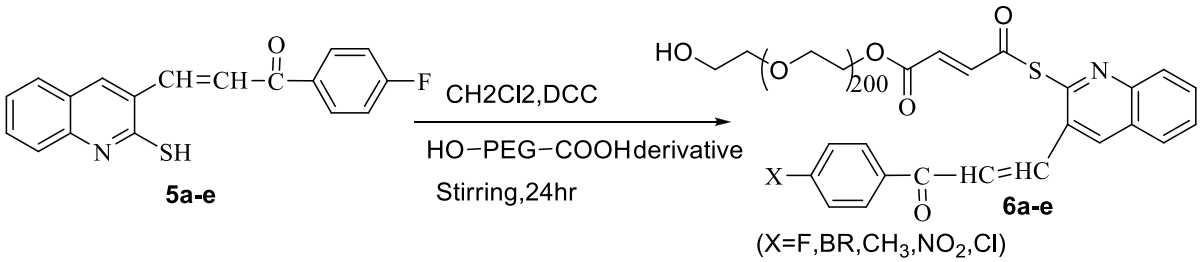
Polyethylene glycol (28.0 mmol, 5ml 200 gm/mol) was disslove in 20 ml of dry CH_2Cl_2. To this solution was added THF containing maleic anhydride (56.0 mmol, 0.54 mg) and pyridine (56.0 mmol, 0.46 mL). The mono acid derivative of poly(ethylene glycol)$_{200}$ was used without purification. **Scheme 4**



Scheme 4: Synthesis of hydroxycarboxy polyethylene glycol (HO-PEG$_{200}$COOH)

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

The mono acid derivative of hydroxycarboxy PEG (HO-PEG$_{200}$ COOH) (28.0 mmol) was activated with 1:2 moler equivalent of 1-(4-fluorophenyl)3-(2-mercaptoquinoline-3-yl)prop-2-en-1-one (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 dissolve in 15ml 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. The resin was dried in vacuum for IR, ^{1}HNMR, ^{13}CNMR and mass characterisation. 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. **Scheme 5** 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 MH_Z $CDCl_3$) □□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 5: Synthesis of PEGylated-1-(4-fluorophenyl)3-(2-mercaptoquinoline-3-yl)prop-2-en-1-one.

2.5.2. Synthesis of PEGylated-1-(4-bromophenyl)-3-(2-mercaptoquinolin-3-yl)prop-2-en-1-one (6b):

Yield: 89%, IR (KBr/λ_{max}cm^{-1}), 3407 (-OH-PEG), 2916 (CH_2-PEG), 1644 (-C=O, -PEG) ,1100 (-CH_2-O-CH_2) cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$ /DMSO-d6) (ppm) 0.84 (s, 1H, OH-PEG Polymer); 1.28-4.41 (m, CH_2-PEG-Polymer), 6.84-6.86 (d, 1H, J=8.00, -CH), 7.24-7.33(m, 2H, Hb, Hc), 7.35-7.41 (m, 2H, Hb, Hc), 7.43-7.46 (m, 4H, Ar-H), 7.54-7.56 (d, 1H, J=8.00, -CH), 7.62 (s 1H, Ar-H), ^{13}C NMR (200 MH_Z $CDCl_3$) **δ** 60.30, 63.64, 69.76, 69.80, 69.85, 69.87, 69.90, 72.40, 122.35, 124.55, 127.85, 128.00, 128.98, 129.14, 130.96, 132.32, 133.82, 135.75, 137.05, 140.01, 145.00, 147.04, 148.91, 169.12, 177.31, 187.42, 189.99, Mass Spectrum m/z 652 M^+

2.5.3. 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 MH_Z $CDCl_3$) **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^+

2.5.4. 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^+

2.5.5. Synthesis of PEGylated-1-(4-chlorophenyl)-3-(2-mercaptoquinolin-3-yl)prop-2-en-1-one (6e):

Yield: 90%, 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) 1.05 (s, 1H, OH-PEG Polymer); 1.21-4.63 (m, CH_2-PEG-Polymer), 7.08-7.10(d, 1H, J=7.96, -CH), 7.12-7.15 (m, 2H, He), 7.13-7.22 (m, 2H, Ar-H), 7.25-7.51 (m, 4H, Ar-H), 7.51-7.71 (d, 1H, J=7.6, -CH), 8.19 (s, 1Ha, Ar-H), ^{13}C NMR (200 MH_Z, $CDCl_3$) **δ** 60.00, 63.24, 69.50, 69.55, 69.58, 69.61, 72.11, 100.65, 122.95, 125.00, 128.25, 128.50, 129.78, 129.94, 131.56, 132.98, 134.12, 136.05, 137.75, 140.22, 147.58, 149.00, 167.20, 177.92, 187.92, 190.00, Mass Spectrum m/z 572 M^+.

Table 3: Physical data of PEGylated quinolines (6a-e)

Compound	6a	6b	6c	6d	6e
Density (*d*) in Cm^3	1.127	1.118	1.123	1.151	1.162

3. Biology

***In vitro* anti-bacterial activity**

3.1 General screening procedure:

All the newly synthesized compounds were screened *in vitro* for their antibacterial 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 40-50ºC 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 37ºC 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-quinoline based chalcone 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: Antimicrobial activity of compounds at 100µg/50µg/25µg

Sr. No.	Compound No.	Diameter of zone of inhibition in mm					
		E. coli			*S. aureus*		
		25 µg/ML	50 µg/ML	100 µg/ML	25 µg/ML	50 µg/ML	100 µg/ML
1	6a	7	12	25	4	12	31
2	6b	6	15	20	5	10	25
3	6c	5	14	21	5	9	19
4	6d	9	19	30	5	9	30
5	6e	4	16	24	4	10	26
Std.	Ampicillin	18	28	31	14	17	30
Std.	Doxycycline	15	26	30	15	18	29
Solvent	DMSO	-	-	-	-	-	-

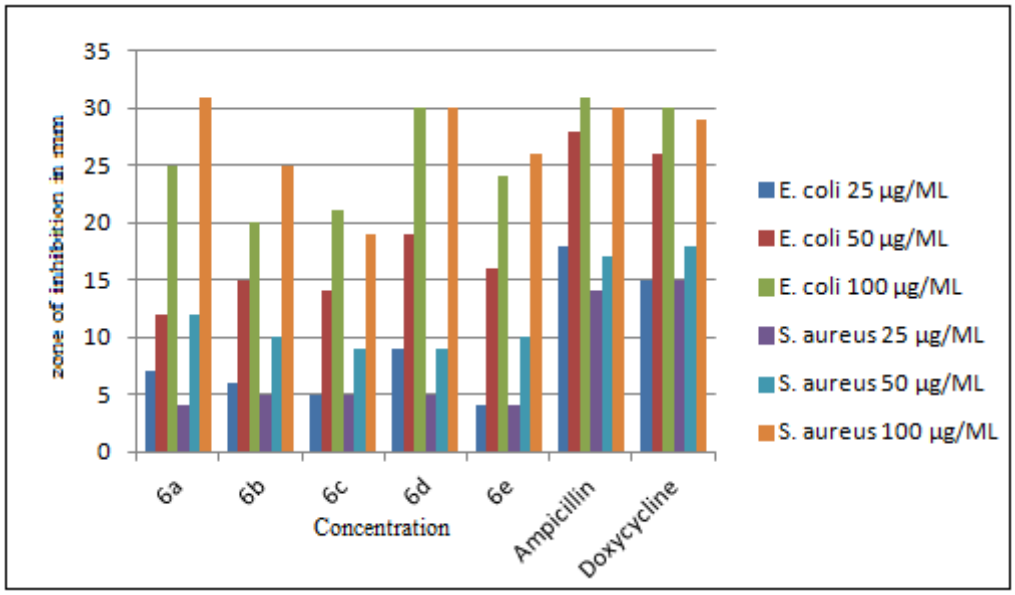


Figure 1: Inhibition in the growth of the *E. coli* and *Staphylococcus aureus* at 25, 50 and 100µg/mL compared with untreated control, standard Ampicillin and Doxycycline

4. Results and discussion

We prepared high value quinoline derivatives adorned with unsaturated functionalities at C-3 position within the same molecular framework by classical method To develop high value quinoline hybrids incorporated with chalcone, derivatives approaches Thiol specific- site PEGylation and Synthesis of Hetero-Bifunctional series of compounds. A new class of surface functionalized PEGylated--quinoline based chalcone were synthesized.All PEGylated--quinoline based chalcone were characterized by CHN, elemental analysis, IR, ^{1}HNMR, ^{13}C NMR, Mass spectral analysis and. Target analogs **6a,6b,6c,6d and 6e** 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 **6a, 6b, 6c, 6d and 6e** against these organisms are depicted **Table 4**. The results were compared with Ampicillin and Doxycycline for antibacterial study as standard drugs. **(Figures 1).**

5. Conclusion

In this study, two independent approaches that conjugating quinoline based chalcone with effective thiol site and mounting this passive targeting moieties onto the reactive PEG(200) derivative were adopted to fabricate PEGylated quinoline based chalcone. 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,**6d**, **6a**, and **6b** synthesized compounds are active against *Escherichia coli* whereas **6d** displayed excellent activity. However, it showed moderate sensitivity against all compounds **at** 50 µg/mL. Amongst all compounds, **6a** and **6d** 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 **6b**, **6c** and **6d** at 25 μg/mL tested in the activity. Compounds **6d** and **6a** showed extraordinary activity and remaining compounds showed good sensitivity at 50 μg/mL. At 100 μg/mL, while rest of the compounds exhibited respectable activity against both *Staphylococcus aureus* and *Escherichia coli*

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