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Research Article

SYNTHESIS, CHARACTERIZATION OF NOVEL FURAN BASED POLYMERIC NANOPARTICLES AND THEIR BIOLOGICAL STUDIES

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ABSTRACT

Objective PEGylation (polymeric substance) is clinically proven and attract both scientific and commercial interests, here we emphasize the overcome the drawback of solution phase methods, the five membered ring, imidazolone moiety is present in a wide range of naturally occurring molecules, for example furan is a five membered heterocyclic nucleus which contain oxygen atom as heteroatom having a broad spectrum of biological activity and here we attempt PEG lated product, involved free carbonyl terminal was used for conjugation via condensation. The solid matrix characterized by spectral studies and their biological activity.

Result we prepared a series of PEGlyted 3-(4-Acetyl-phenyl)-5-aryldine-2-furan-2-yl-3,5-dihydro-imidazol-4-one with different aldehydes through Erlenmeyer reaction and condensation methods by using PEG-aldehyde. These newly synthesized compounds were characterized by IR, 1HNMR, MASS, SEM, DLS studies. All final compounds are screened for their antioxidant, anti-inflammatory activities done through by DPPH, Nitric oxide radical scavenging, ferrous ion chelating and hemolytic assay.

Conclusion furan based compounds having imidazolone moiety having greater importance in medicinal chemistry. In this work we attempt to highlight those compounds which show good potential against Biological activity. Of all the compounds 4c, showed to be a potent molecule.

INTRODUCTION

PEGylation of small organic molecule or drugs, protein are conjugated to the distal end of PEG carrier, here PEG's are used as conjugate agent. This PEGylation is to avoid glycol and modified polymer, which plays a vital role in drug delivery, due to morphological behaviour shows good potent against targeted one[1-4]. It increases the solubility in water and chemical stability[5].Generaly macromolecular PEGs may block activity of small active agents at the target dells via steric hindrance. Because of to overcome, the low molecular weight PEGylation (<10,000Da) was employed, which is conjugate chemically, enzymaticaly actively transferred into their target sites, for reasonable attachments are narmally called "prodrug approach".[6-9].To increase the drug load, different types of PEG conjugations are employed such as branched , Forked and multi armed (star like) PEG's for examples like NKTR-102 (PEG-irinotecon) EZN-2208 (PEG-SN38) & NKT R-105(PRG-docetaxel). Branched or "umbrella like" structure, this technology is preferred in protein or enzyme PEGylation but is not applied as frequently with small molecule[10-13]. PEGylation of the most commonly what we used for the anti-inflammatory drug *gentamycin*, antimicrobial drug *amphotercin-B* and *curcumin* which has been used as anti-inflammatory, antioxidant and anticancer effect, shows significant increase in their activity at very lower concentrations compared with their respective parent drugs[14-17].Here we synthesize some furan based imidazolone compounds and adding some melamine groups to increase the activity through Erlenmeyer reaction, condensation reaction, Diels order reaction and all the compounds are screened for antioxidant activity using DPPH radical scavenging, Nitric oxide, Ferrous ion chelating and Haemolytic activity and the compounds are confirmed through Mass, IR,1HNMR, DLS and SEM.

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Chemistry

METERIAL AND METHODS

The entire chemical purchased from Sigma and SD fine chemicals, product obtained are characterized by spectral studies and DLS, SEM, which is obtained from IOE, University of mysore, Mysuru. The PEG-CHO was prepared according Harris and *et al*., procedure. The products were monitored by TLC technique and their melting point by an open capillary method which is uncorrected.

General procedure for the preparation of the compounds (2a-d)

DMF/$POCl_3$mixture was prepared by addition of DMF to $POCl_3$ with constant stirring for 30 minutes maintaining 0-5 °C. To this add substituted oxazolones, Para amino acetophenone (1:1) ratio and catalytic amount of Celite -545 were added and refluxed for 2-hours for maintaining 0°C. The solid was poured into crushed ice allow to settle for few minutes filter and purification done through column chromatography.

General procedure for the preparation of the compound (4a)

The substituted compound(2a)(0.01m)and 1,1'-(methylenedi-4,1-phenylene) bismaleimide (0.01m)in THF was refluxed for 4 hours at 60°C, the un aromatized product called as DA adduct(4a) and it is reversible in nature. Therefore the product further refluxed by adding 2ml of acetic anhydride to offered aromatization. The resultant product was poured in to crush ice-cold water washed, filter and air dried[20].

PEGylation of the compounds 2(a-d)

PEGylation of 3-(4-Acetyl-phenyl)-5-aryldine-2-furan-2-yl-3,5-dihydro-imidazol-4-one compounds were done through by precipitation method[18-19]. According to Raman Dhivya *et al*., and with slight modification, all the substituted3-(4-Acetyl-phenyl)-5-aryldine-2-furan-2-yl-3,5-dihydro-imidazol-4-one compounds are dissolved in chloroform at lab temperature with little amount of KOH pellets and added drop wise into the PEG-CHO which is dissolved in distilled water and stirred vigorously for two hours, then kept it aside for 10 minutes to separate the aqueous layer from organic layer . Aqueous layer and excess of PEG-CHO removed by washing organic layer with distilled water in several times by centrifugation and made to boil (40°C) to evaporate $CHCl_3$, which gave waxy liquid, after cooling becomes a solid.

(Z)-1-(4-acetylphenyl)-2-(furan-2-yl)-4(furan-2-yl-methylene)-1H-imidazol-5(4H) –one (2a) Colour: Yellow solid, Yield: 75%,Mp., 210°C IR (KBr,cm^{-1}); 3160(Ar-H Str),2890(alipha-CHstr),1750(C=O),1060(CNStr);^{1}HNMR (400, MHz, dmso. δ/ppm);2.2 (S,3H,CH_3),6.7-6.83(m,2H,Fu-H),7.1(S,1H,Fu-H), 7.4-7.5 (m,4H, Ar-H,Fu-H) ,7.99(s,-CH=),8-8.1(3H,Fu-H,Ar-H,); ^{13}CNMR (100 MHz. $CDCl_3$,δ/ppm), 26.6,109.9,112, 124.6,129. 8,132.5, 136. 7,141.4, 142.2143, 153.93, 169. 6, 198.5; ESIMS (M/Z); 343(M) $^+$.Anal. cald. for$C_{20}H_{14}N_2O_4$; C,69.36: H,4.04, found: C,69.32:H,4.01(Z) 1- (4-acetylphenyl) -4-benzylidene-2-(furan-yl)-1H-imidazol-5 (4H)-one(2b) Colour; Yellow solid, Yield:81%;Mp.,98°C;IR(KBr,cm-^{1});3130 (ArHStr), 2089 (alipha CHstr), 1759 (C=O),1068 (CNStr);^{1} HNMR (400, MHz, dmso. δ/ppm) ;3.15 (S,3H,CH_3), 6.8(S,1H,Fu-H), 7.25-7.8 (m.10H,,Ar-H,-CH=,Fu- H),7.65 (t,1H,F-H), 8.17 (m,1H,Fu-H),^{13}CNMR; (100MHz.$CDCl_3$, δ/ppm), 26. 5, 56, 109, 113,124, 128,129.7, 130.5,132. 2,135, 136.5, 142,144.5, 197.5; ESIMS (M/Z); 356(M)$^+$ .Anal. calcd. For C22 H_{16}, N_2 O_3; C, 71.1:H, 4.49, forund: C,74;H,4.45.

(Z)-1-(4-acetylphenyl)-2-(furan-2y)-4(3,4,5-trimethoxy benz ylidene) -1H-imidizol-5(4H)-one(2c) Colour: bright yellow solid ,Yield: 80%, Mp., 170°C,IR (KBr,cm^{-1}) ;3134(Ar-H Str), 2894 (alipha CHstr), 1760 (C=O),1060 (CNStr); ^{1}HNMR (400,MHz,dmso.δ/ppm); 2.2(S,3H,CH3) ,3.83.9 (m,9H,OCH_3), 6.8(S,1H,FuranH),7.25-7.5(m,4H,Ar-H),7.5-8.1 (5H,Fu-H,Ar-H,-CH=)-;^{13}CNMR-(100MHz.$CDCl_3$,δ/ppm),27.5,56, 104, 109, 116.8,120.5,125.7,127.4,132.21,142,55.9,160.6,198.5; ESIMS (M/Z);431(M)$^+$.Anal. cald. for $C_{25}H_{22}$ N_2O_6; C,67.26: H,4.9,found:C,67.23:H,4.6.

(Z)-1-(4-acetylphenyl)-2-(furan-2-yl)-4- (4-methoxybezyliden e)-1H-imidazole-5(4H)-one (2d) Colour: yellowsolid, Yield: 81%,Mp.,145°C,IR (KBr,cm^{-1}); 3160 (Ar-H Str), 2900 (alipha CHstr), 1760(C=O), 1098 (CNStr); ^{1}HNMR (400, MHz, dmso. δ/ppm); 2.2 (S,3H,CH_3), 3.9 (S,3H,OCH3), 6.8(S,1H,F-H), 6.95-7.2,(m,4H,Ar-H),7.85-8.1(7H,Fu-H,Ar-H,-CH=) ^{13}CNMR (100MHz .$CDCl_3$,δ/ppm) 27.5,56.3,104,109, 116,120.5,124. 7,127.5,132, 142,155.9, 160.6, 199.5; ESIMS (M/Z); 373 (M)$^+$ Anal. cald. for: $C_{23}H_{18}N_2O_4$; ,71.42: H,4.65, found: C,71.12:H,4.63. (Z)-1-(4-acetylphenyl)-2-(furan-2-yl)-4(furan-2-yl-methylene)-1H-imidazol-5(4H)-one and 1, 1'-(methy lenedi -4,1-phenylene) bismaleimide (A-B)adduct (4a) Colour: Yellow solid, Yield: 90%MP>275°C.IR(KBr,cm^{-1});3167(Ar-HStr) 3030 (alipha-CHstr),1709 (C=O), 1030(CN Str);^{1} HNMR (400, MHz, $CDCl_3$.δ/ppm); 2.5(CH_3),3.9(CH2=CH2-)6.9(H>C=C<H) 6.5-8.2(ArCH). ESIMS (M/Z); 996(M) $^+$.

Spectral studies of PEGylated compounds

PEGylated compound of 3a ((Z)-1-(4-acetylphenyl)-2-(furan-2-yl)-4 (furan-2-yl-methylene) -1H-imidazol-5 (4H)-one). IR (KBr, cm^{-1}); 3160(Ar-H Str), 2882 (alipha-CHstr), 1649(C=O), 1063 (CNStr). ^{1}HNMR (400, MHz, $CDCl_3$.δ/ppm); Peak at 3.648 (PEG backbone), 2. 32(-CH_2=CH_2 -C=O), 2,53(O-CH_2-CH_2),7.2(Ar-H). ESIM (M/Z); 4717(M) $^+$

PEGylated compound of 3b ((Z)-1-(4-acetylphenyl)-4-benzylidene-2-(furan-yl)-1H-imidazol-5(4H)-one-) IR (KBr, cm^{-1}); 3160 (Ar-HStr), 2882(alipha-CHstr), 1649 (C=O), 1063 (CN Str).^{1}HNMR (400, MHz, $CDCl_3$. δ/ppm); Peak at3. 648 (PEG backbone), 2.32 (-CH_2=CH_2-C=O), 2, 53 (O-CH_2-CH_2),7.2 (Ar-H); ESIM(M/Z); 4737(M) $^+$

PEGylated compound of 3c ((Z)-1-(4-acetylphenyl)-2-(furan-2y)-4(3,4,5-tri methoxy benzylidene)-1H-imidizol-5(4H)-one-) IR(KBr,cm^{-1});3160(Ar-HStr), 2882(alipha- CHstr),1649(C=O), 1063(CNStr)^{1}HNMR (400,MHz,$CDCl_3$. δ/ppm); Peak at 3.648 (PEG backbone),3.5(OCH_3)2.34(CH_2=CH_2-C=O),2,53(O-CH_2-CH_2),7.5(Ar-H); ESIMS (M/Z); 4916(M) $^+$.

PEGylated compound of 3d ((Z)-1-(4-acetylphenyl)-2-(furan-2-yl)-4-(4-methoxy bezylidene)-1H-imidazole-5(4H)-one) IR (KBr, cm^{-1});3160(Ar-H Str), 2882 (alipha-CHstr), 1649(C=O),1063(CN Str) ^{1}HNMR (400,MHz,$CDCl_3$. δ/ppm); Peak at3.648 (PEG backbone),3.9(OCH3),2.34(-CH_2=CH_2-C=O),2,53(O-CH_2CH_2),7.2(Ar-H); ESIMS (M/Z); 4796(M) $^+$.

DA adduct (A-B type)-PEG (4b)

IR (KBr,cm^{-1}); 3160 (Ar-HStr), 2882 (alipha-CHstr), 1646 (C=O), 1063(CN Str) ^{1}HNMR (400,MHz,$CDCl_3$.δ/ppm); Peak at 3.648 (PE G backbone),2.34(-CH_2=CH_2-=C=O) 2.53(O-CH_2-CH_2),7.2(Ar-H);

Biological activity

Antioxidant assay

Dpph Radical Scavenging Assay

DPPH radical scavenging activity was done by the method of Shone stall, (1998) with little modifications. Briefly, one ml of DPPH solution (0.1 mM in methanol) was incubated with gradient concentrations (20µg/ml to 100µg/ml) of the synthetic Imidazolone compounds, shaken and incubated for 30 min at room temperature and absorbance was read at 517nm against a blank. BHT was used as reference compound. The radical scavenging activity was measured as decreases in the absorbance of DPPH and calculated by using the following equation. Radical scavenging potential was expressed asIC_{50} value, which represents the sample concentration at which 50% of the DPPH radical were scavenged. Scavenging effect= [1-sample absorbance (517nm)/control absorbance (517nm) ×100]

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was performed by the method of (Marcoci et.al, 1994), with minor modifications. Nitric oxide was generated from Sodium nitroprusside, in aqueous solution at 7.3 $_P$H, spontaneously generated nitric oxide reacts with oxygen to produce nitrite ions that can be estimated by the Griess reagent Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium Nitroprusside (5mM) in Phosphate buffer saline was mixed with the synthetic imidazolone compounds are incubated at room temperature for 60 min.

The sample from the above was reacted with Griess reagent (1% sulphanilamide, 2%H_3PO_4 and 1% naphthalene diamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and coupling with naphthalene diamine was read at 546 nm and referred to the absorbance of BHT treated into the same way with Griess reagent. The radical scavenging activity was measured using the equation described for DPPH radical scavenging activity.

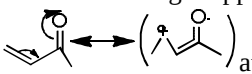
Ferrous ion chelating assay

Ferrous ion chelating ability was measured according to Gordon M.H. 1990 *et al*, method 29. For the assay, three sets of test tube were taken. One tube was taken as control to this $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1 ml by adding distilled water. For the second tube, EDTA (40 mM), $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1 ml by adding distilled water. For the third one, test compounds imidazolone with concentrations 20, 40, 60, 80 and 100 µg, $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1 ml by adding distilled water. The tube was incubated for 10 min at 20 ºC and the absorbance was read at 700 nm and ion chelating ability was calculated. The anti-oxidant activity of all the compounds was compared with that of BHT. Radical scavenging activity was expressed as percentage;

Indirect and direct study of haemolytic assay

A semi quantitative indirect haemolytic assay (BOMAN and KALETTA, 1957) was employed. Briefly, packed human erythrocytes, egg yolk and phosphate buffer saline were mixed (1:1:8 v/v).one ml of this suspension was incubated with 20µg enzyme for 10 min at 37°c.The reaction was stopped by adding 10 ml of ice cold phosphate buffer saline and centrifuged at 4°c for 10 min at 800xg.The amount of haemoglobin released in the supernatant was measured at 540nm.

RESULT AND DISCUSSION

The compounds 2(a-d) are synthesized through Erlenmeyer reaction, condensation and DA (AB) reaction, made to react with PEG-CHO. These are waxy solid matrix and easily soluble in water and organic solvents, the size of these substances from 1nm to 99 nm range. The spectral data of IR absorption band between, 1020-1090cm^{-1}, 1709cm^{-1},2770-2900cm^{-1},3130-3314cm^{-1} due to C=N ,C=O ,CH aliphatic and Aromatic C-H stretching, and of PEGlyted compound the -C=0 stretching appear at lower frequency[1] due to  at 1649cm-^{1}, instead of 1709cm^{-1}.

^{1}HNMR shows that the shift δ2.2- 2.5,-CH3, δ3.2-3.9(-OCH_3), δ6.5-8.1(Ar-H, Furan-H). PEGylated compound of ^{1}H NMR spectrum shows peak atδ3.648 (PEG backbone), 2.32 (-CH_2=CH_2-C=O), 2, 53(O-CH_2-CH_2), 7.2(Ar-H). For the indication of the end group attachment of drug to the polymer linearly. Mass spectra showed agreeable value for proposed structures**.** I.e. the mass of the original one is increased by PEGylation which was the presence of end group attachment of the drug to the polymer linearly.

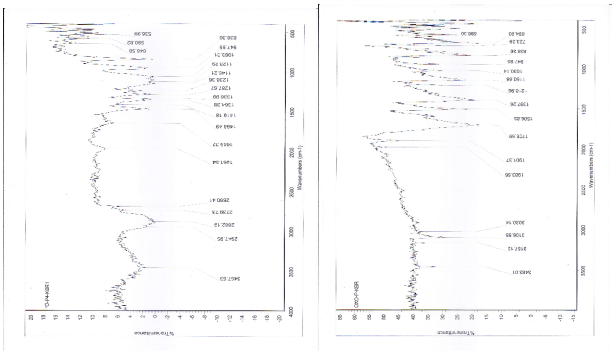


Figure1 with (a) and without (b)PEGylation

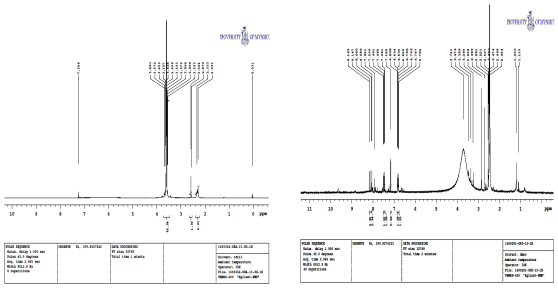


Figure 2 with (3a) and without (b) PEGylation of ^{1}HNMR spectra.

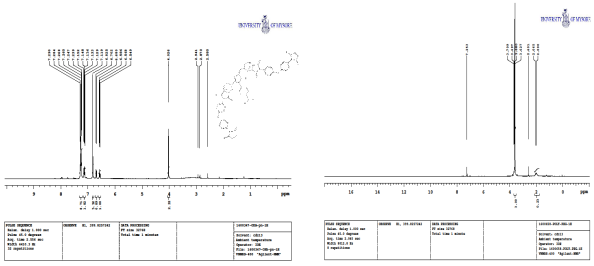


Figure 3 ^{1}HNMR spectrum of compound 4a and 4b.

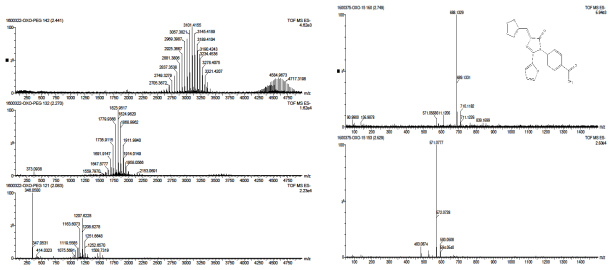
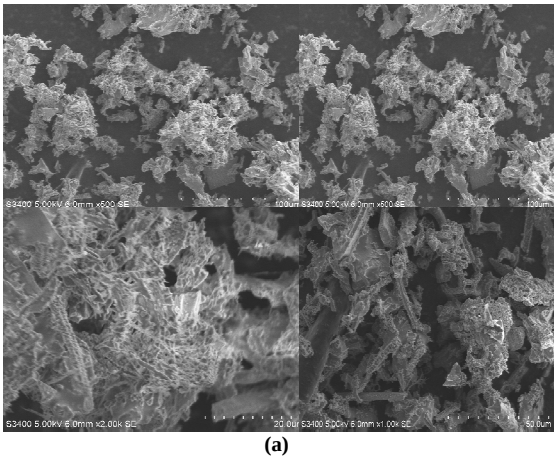


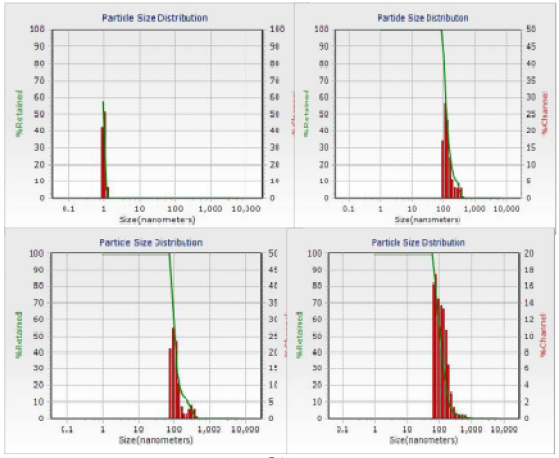
Figure 4 Mass spectrum of the compound 3a and 2a.

Surface morphology

SEM images and histogram of the particle size distributions of the PEGylated compound are shown in fig.5 the average particle size obtained 1 to 99 nm respectively.



(a)



(b)

Figure 5 SEM images (a) and (b) DLS of PEGylated compounds.

In the figure.6, 7, 8 the antioxidant activity of PEGylated compounds 3c, 3d, 4b and followed by 4a, were showing significant scavenging activity indicating the potency of the molecules. Some of the PEGylated compounds are accelerate effectively on the biological activity due to blockage of targeted moiety which poorly executes the biological activity or there is an increase in the activity due to the dosage or of the proper orientation of targeted groups of the compounds. Electron donating group like OCH_3 and steric hindrance of trimethoxy groups present in 3d and 3c were affected for the good activity.

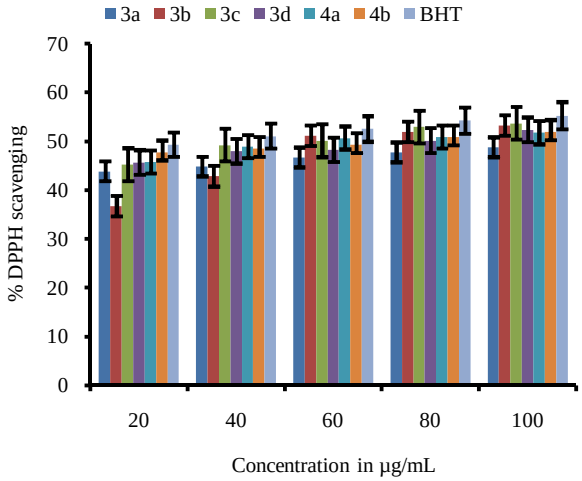


Figure 6 DPPH scavenging assay of compounds 3a-4b

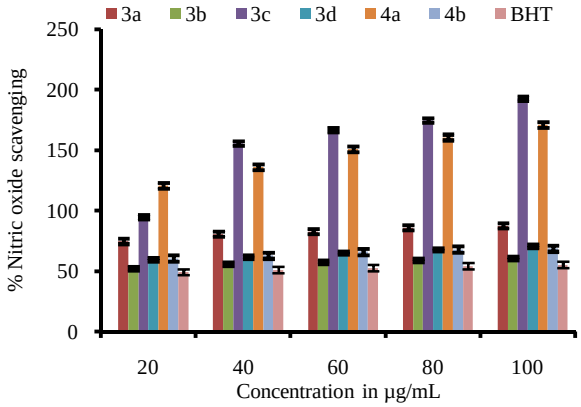


Figure 7 Nitric Oxide radical scavenging assay of 3a-4b

This could be purely depending on the morphological behaviour of the compounds. But in the case of 4a the melamine group responsible for the better antioxidant activity.

Among the five PEGylated nano compounds of Haemolytic assay, 3c was found to be more potent in inhibiting Phospholipase A2 enzyme from the Russel's viper snake venom with an IC_{50} value of 30.66µgs.

followed by 3b with an IC_{50} value of 85.34 µg.3d and3a are showing almost same activity with an IC_{50} value of 111.66µgs and 113.31µgs.Among all the compounds 4b was found to be less potent with an IC_{50} value of 139.48µgs.

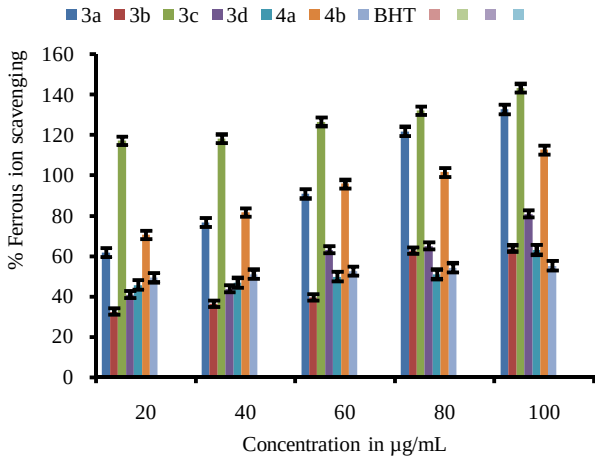


Figure 8 Ferrous ion scavenging assay of compounds 3a-4b

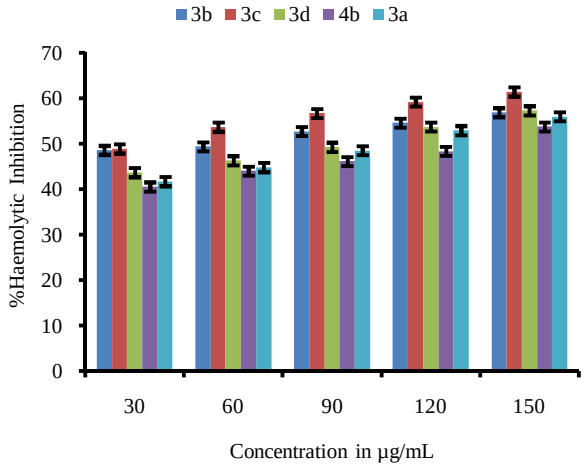


Figure 9 Haemolytic assay of PEGylated compounds.

CONCLUSION

All synthesized compounds are screened for anti oxidant and Haemolytic assay. In that the compound 3c become good potent molecule, because of having furan and imidazolone moeity and steric hindrance of tri methoxy groups present in that molecule, followed by 3b were showed to be potency in the above Biological activity. Moreover size and mass is not affected for the biological assay.

Authors's contributions

Dakshayini and devaraju designed research dakshayini performed the research; dakshayini, mallu and Devaraju analysed spectral data; Rekha and Ranjini analyzed biological data. Dakshayini and Rekha wrote the paper. All the authors read and approved the final manuscript. Competing interests
The authors declare that they have no competing interests

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