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Designing, Synthesis, Characterization And In Vitro Antibacterial Activity Evaluation Of New Heterocyclic 4-Methyl-2h-Benzo[H]Chromen-2-One Based Spiro Derivatives

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The present study explain the synthesis of some new heterocyclic spiro Substituted/unsubstituted -3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonothioyl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione, Substituted/unsubstituted--3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonyl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione, Substituted/unsubstituted--3'-(4-methyl-2-oxobenzo[h]quinolin-1(2H)-yl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione ,derivaties (8 a-c,9 a-c,10a-c) initially the 4-methyl-2H-benzo[h]chromen-2-one synthesized as condensation product of of α-naphthol and ethyl acetoacetate.which further react with urea, thiourea and hydrazine hydrate obtained amine. The precursor imine has been formed by the reaction of synthesized amine and isatin.the targeted spiro derivatives with good yield obtained by the cyclization of imine using thioglycolic acid. The structure of synthesized spiro derivatives established by the U-Visisble, IR-KBr, 1HNMR and Mass. The synthesized derivatives show excellent to moderate antimicrobial activity.

Keywords: ethyl acetoacetate, isatin, urea, thioglycolic acid, thourea, pyridine.

Introduction:

Spiro compounds are the most sought-after molecules in modern therapeutics and are extensively employed in chiral ligands, organometallic complexes, organic LED alongwith their ubiquitous presence in various natural products and derivatives. 3substituted thalide and

their associated spirocyclic derivatives demonstartate exceptional pharmacophoric attributes such as tyrosinase inhibition[1-2], amoebicidal [3-4], antioxidant[5-6] and antitumor [7-8].

Spiro2,3 dihyroindole derivatives exhibit tremendous biological activities particularly anticancerous activities[9-12]. The presence of two components may be the reason for the excellent activity against the cancer disease[13-15]. The demand for efficient therpaeutics to avoid the hazards of long-term chemo in treataing colorectal cancer urged the exploration of newer molecules to combat the disease[16-18]. The spiro compounds were first developed by VonBayer in 1900 and was initially known as spirocyclane[19-22]. It belongs to bicyclic hydrocarbon bearing a single carbon bond. Since the carbon associated with the spiro linkage is tetrahedral ot causes the ring planes to align almost parallel to each other. The asymmetry of the molecule is keenly responsible the unique biological activities [23-25] which is due to the chirality of the spiro carbon atom[26-28]. Thus, the spiro compounds and its aderivatives continue to awaken new vistas in drug research[29-30].

Indolin 2-ones or isatin derivatives show anticancerous properties by inhibiting histone deacylase HDACS, carbonic anhydrase epridermal growth factor receptor tyrosin kinase EGFr-TK and tubulin. Isatin also depict antibacterial, antidiabetic, anticonvulsant antituberculosis antiviral activities[9-12]. They are employed as corrosion inhibitors fluroscent sensorsor probes and dyes. There is significant demand for development of new small molecules that can effectively target colorectal cancer, thereby reducing the risks associated with the long-term use of conventional chemo drugs. The isatin motif basically the cycoprpane ring structure provides for the easy accessibility of the plasma membrane enhance potency and metabolic stability of the therapeutic drug

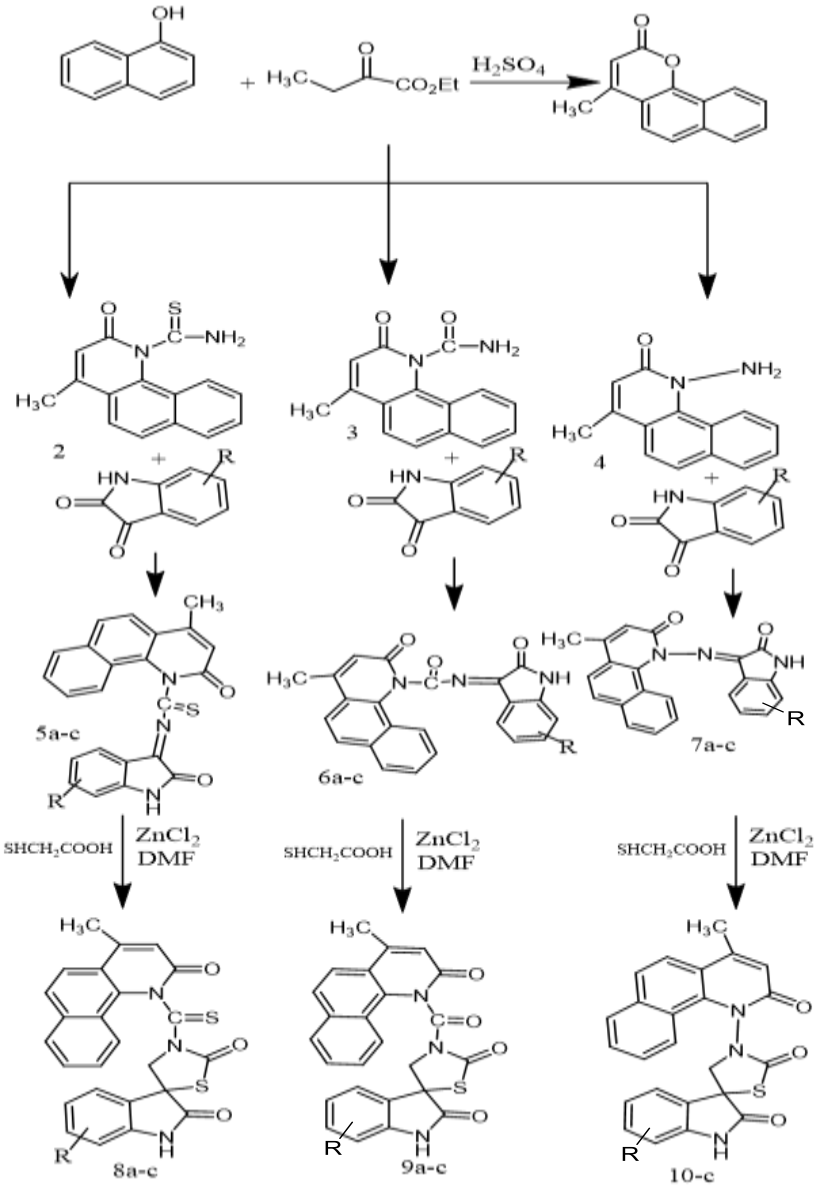
Heterocyclic compounds are ubiquitous and impose enormous impact on human health[31-32] . As such they are important constituent in many natural compounds like vitamins hormones, amongst them Thiazolidine -4-one holds a distinctive place . It is specially of huge significance in pharmaceutical and drug related research and explorations. amongst the various possible ways of synthesizing the molecule one method is through thee formation of a Schiff base by condensing amine with an aldehyde /ketone followed by its conversion into a cyclic moleculevia TGA

The low cost and excellent accuracy involved in the density functional theory or DFT technique has garnered special attention in drug research It aces in predicting the molecular geometries and molecular energies and the mechanism. It displays precisely the 3Dimensional structure and the related active sites of the molecule by simulating and representing biomolecular structure and the surrounding media incumbent for any successful reaction. Thus, it allows for an easy monitoring of the drug target interactionsand their related binding affinities. greatly reducing the the timw erequired in finding receptors enzymes proteins which fit the bill [33-34].

It also aids in emanicipating the desired effect on a particular target and also tends to enhance solubility stability and selectivity of chemicals. It also provides for the assesemment of the potential toxicity of the developed compoundsthereby considerably reducing the hazard pose to the patient. Additionally, it helps when trying to guess which compounds will have the

desired effect on a specific biological target. It helps with enhancing the pharmacologically significant solubility, stability, and selectivity of chemicals [35]. This technique aids in the development of safer and more effective medications for patients by predicting the potential toxicity of therapeutic molecules and identifying undesirable interaction sites [13-15]. In developed nations, hypertension is becoming more common; this condition is associated with an increased risk

SCHEME-1



Chemistry: The order of prepration depicted in scheme-1 was fallow to synthesized the spiro- indoline-thiazolidine-dione derivatives. The **4-methyl-2H-benzo[h]chromen-2-one was syhthesized by** Pechmann reaction and thiourea, urea, hydrazine hydrates to furnish amine which converted into Schiff base by the condensation of amine and isatin, and their structur establishment using the the infrared-vmax per cm-KBr and PMR-Deuterated chloroform-300 MHz δ in ppm with provided spectral data : 1635 (C=O), 3051 (Aromatic, CH) and: 6.95-8.71-9H,multipilate of aromatic ring and 3H at 1.40 (-CH3). final spiro derivatives spiro Substituted/unsubstituted -3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonothioyl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione 8 a-c, Substituted/unsubstituted--3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonyl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione 9 a-c, Substituted/unsubstituted--3'-(4-methyl-2-oxobenzo[h]quinolin-1(2H)-yl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione10a-c formed as cyclisation of imine by thioglycolic acid and in trace amount of $ZnCl_2$ in DMF solvent. . The structure of formed spiro-derivatives determines by spectral data the infrared-vmax per cm-KBr: data: 1738 (C=O), 3048 (Aromatic, CH) ,618(CS) and PMR-Deuterated chloroform-300 MHz δ in ppm 6.85-8.74-9H, multipilate of aromatic ring and 3H at 1.1 (-CH3).

Experimental Section:

All the chemical used were of laboratory grade. The meltig points of prepared derivatives were determine by Electro Thermal apparatus using fused capillary. the progress reactions and purity of derivative was examined by TLC using silicagel plates of 0.5 mm thickness as stationary phase and combination of chloroform and ethanol in various ratio combination. The iodine chamber was used for the development of slides. The 1HNMR spectra were recorded in CDCl3 and DMSO on Bruker NMR spectrophotometer at 400 MHz.the internal standard tetramethylsilane was used and chemical shift value (δ) were given in part per million(ppm). The following instruments were used: Jasco FTIR-470 spectrophotometer (KBr) with diffuse reflectance method; MS-JEOL SX102 Mass spectrometery by using Argon/Xenon (6Kv, 10mA) as the FAB gas and m-nitro benzyl alcohol (NBA) as the matrix.

1. The synthesis of 4-methyl-2H-benzo[h]chromen-2-one I:

A (0.1 mole) of α-naphthol and ethyl acetoacetate (0.1 mole) with 40 ml conc. sulphuric acid was stirred for 0.5h. The resulting dark green solution was cooled and poured over crushed ice (250gm). The obtained product was filtered off and washed repeatedly with water and dried at 100°C. The anhydrous coumarins thus obtained, were insoluble in methanol and recrystallised with ethanol.

Yield 76 %; mp 93°C; Mol. Wt: 210.23 nal. Calcd. for $C_{14}H_{10}O_2$, C, 79.98; H, 4.79 found:C, 79.93; H, 4.73,IR (KBr): 3063 (C-H, str., Aryl), 2867 (C-H, str.), 1675 (C=O, str.), 1643 (C=N, str.), 1560 (C=C,str., Aryl), 1158 (C-O, str.); 1 H NMR (CDCl 3 /DMSO-d 6) (300 MHz): δ7.53-8.05 (m, 13H, Ar-H), 1.83 (s, 3H, CH 3), 4.02(s,1H,CH=C-CH_3)..

2. The synthesis of 4-methyl-2-oxobenzo[h]quinoline-1(2H)-carbothioamide:
A (0.02 mole) of 4-methyl-2H-benzo[h]chromen-2-one and thiourea (0.02 mole) in pyridine (30 ml) was heated under reflux on a sand-bath for 6 hours. Subsequently, the reaction mixture was poured into ice-cold water (100 ml) containing conc. hydrochloric acid (10 ml). A solid started to separate out which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying in a vacuum desiccator, it was recrystallised from ethanol.

Yield 72 %; mp 96 – 97°C; Mol. Wt: 268.33 Anal. Calcd. For $C15H_{12}N_2OS$ C, 67.14; H, 4.51; N, 10.44 found: C, 67.11; H, 4.47; N, 10.41,IR (KBr): 3060 (C-H, str., Aryl), 2861 (C-H, str.), 1679 (C=O, str.), 1640 (C=N, str.), 1563 (C=C,str., Aryl), 1354 (C-N, str.), 1013 (C=S, str.), 3320 per cm (-NH2); 1 H NMR (CDCl 3 /DMSO-d 6) (300 MHz): δ7.60-8.07 (m, 13H, Ar-H), 1.89 (s, 3H, CH 3), 4.26(s,1H,CH=C-CH_3)..

3. The synthesis of 4- methyl-2-oxobenzo[h]quinoline-1(2H)-carboxamide:
A (0.02 mole) of 4-methyl-2H-benzo[h]chromen-2-one and urea (0.02 mole) in pyridine (30 ml) was heated under reflux on a sand-bath for 6 hours. Subsequently, the reaction mixture was poured into ice-cold water (100 ml) containing conc. hydrochloric acid (10 ml). A solid started to separate out which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying in a vacuum desiccator, it was recrystallised from ethanol.

Yield 59 %; mp 89 – 80°C; Mol. Wt:252.27; Anal. Calcd. for $C_{15}H_{12}N_2O_2$, C, 71.42; H, 4.79;
N,11.10 found:C, 71.35; H, 4.74; N, 11.04, IR (KBr): 3068 (C-H, str., Aryl), 2863 (C-H, str.),
1672 (C=O, str.), 1640 (C=N, str.), 1563 (C=C, str., Aryl), 1359 (C-N, str.);3310(N-H, -NH2),
1 H NMR (CDCl 3 /DMSO-d 6) (300 MHz): δ7.70-8.09 (m, 13H, Ar-H), 1.88 (s, 3H, CH 3),
4.10(s,1H, CH=C-CH_3).

4. The synthesis of 1-amino-4-methylbenzo[h]quinolin-2(1H)-one:
A (0.02 mole) of 4-methyl-2H-benzo[h]chromen-2-one and Hydrazine Hydrate (0.02 mole) in pyridine (30 ml) was heated under reflux on a sand-bath for 6 hours. Subsequently, the reaction mixture was poured into ice-cold water (100 ml) containing conc. hydrochloric acid (10 ml). A solid started to separate out which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying in a vacuum desiccator, it was recrystallised from ethanol.

Yield 81 %; mp 116 – 117°C; Mol. Wt: 224.26 Anal. Calcd. for C14H12N2O, C, 74.98; H, 5.39; N, 12.49 found: C, 74.93; H, 5.34; N, 12.47,IR (KBr): 3074 (C-H, str., Aryl), 2880(C-H, str.), 16790(C=O, str.), 1648 (C=N, str.), 1567 (C=C,str., Aryl), 1350 (C-N, str.);1029(N-N),3381(N-H,NH2), 1 H NMR (CDCl 3 /DMSO-d 6) (300 MHz): δ7.50-8.09 (m, 13H, Ar-H), 1.88 (s, 3H, CH 3),4.12(s,1H,CH=C-CH_3).

5. The synthesis of (E)-N-(substituted-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h] quinoline-1(2H)-carbothioamide:

A of each 0.02 mole 4-methyl-2-oxobenzo[h]quinoline-1(2H)-carbothioamide and substituted-isatin in pyridine (30 ml) was heated under reflux on a sand-bath for 6 hours. Subsequently, the reaction mixture was poured into ice-cold water containing conc. hydrochloric acid (10 ml). A solid started to separate out which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying in a vacuum desiccator, it was recrystallised from ethanol.

5a. (E)-N-(5-chloro-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carbothioamide

Yield 78 %; mp 126 – 127°C; Mol. Wt: 430.89 Anal. Calcd. for $C_{23}H_{13}ClN_3O_2S$, C, 64.11; H, 3.04; Cl, 8.23; N, 9.75 found:C, 64.06; H, 3.01; Cl, 8.14; N, 9.71; IR (KBr): 3031 (C-H, str., Arom), 1426 (C=S, str.), 1678(C=N),2855 (C-H, str.), 1635 (C=O, str.),1540 (C=C,str., Arom), 1036 (C-N,str.),615 (isatin-Cl),2832 (CH_3-Arom),3234(N-H); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.4(s,3H,-CH_3),3.1 (s,1H,-CH-CO-N),2.2 (s,1H,NH), 6.95-8.71 (m,9H,aromatic).

5b. (E)-N-(5-bromo-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carbothioamide:

Yield 67 %; mp 95 – 96°C; Mol. Wt: 475.34 Anal. Calcd. for $C_{23}H_{13}BrN_3O_2S$ C, 58.12; H, 2.76; Br, 16.81; N, 8.84 found: C, 58.08; H, 2.70; Br, 16.77; N, 8.80; IR (KBr): 3036 (C-H, str., Arom), 1430 (C=S, str.), 2851(C-H, str.), 1633(C=O, str.), 1670(C=N),1546 (C=C,str., Arom), 1030 (C-N,str.),623 (isatin-Br),2835 (CH_3-Arom),3238(N-H); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.3(s,3H,-CH_3),3.4 (s,1H,-CH-CO-N),2.5(s,1H,NH), 6.91-8.70 (m,9H,aromatic).

5c. (E)-N-(5-hydroxy-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carbothioamide: Yield 58 %; mp 109 – 110°C; Mol. Wt: 412.44; Anal. Calcd. for $C_{23}H_{14}N_3O_3S$, C, 66.98; H, 3.42; N, 10.19 N, 5.12 found: C, 66.93; H, 3.35; N, 10.15;
IR (KBr): 3045 (C-H, str., Arom), 1438 (C=S, str.), 2845(C-H, str.), 1673(C=N), 1644(C=O, str.),1553 (C=C,str., Arom), 1025 (C-N,str.),3623 (isatin-OH),2830 (CH_3-Arom),3245(N-H); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.8(s,3H,-CH_3),3.1 (s,1H,-CH-CO-N),2.1(s,1H,NH), 6.82-8.73 (m,9H,aromatic),3.02 (1H, s, isatin-OH)

6.The synthesis of (E)-N-(substituted-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carboxamide:

A 0.02 mole of each 4- methyl-2-oxobenzo[h]quinoline-1(2H)-carboxamide and substituted-isatin (0.02 mole) in pyridine (30 ml) was heated under reflux on a sand-bath for 6 hours. Subsequently, the reaction mixture was poured into ice-cold water containing conc. hydrochloric acid. A solid started to separate out which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying in a vacuum desiccator, it was recrystallised from ethanol.

6a. (E)-N-(5-chloro-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carboxamide: Yield 63 %; mp 93 – 94°C; Mol. Wt: 414.83 Anal. Calcd. for $C_{23}H_{13}ClN_3O_3$ C, 66.60; H, 3.16; Cl, 8.55; N, 10.13 found:C, 66.54; H, 3.11; Cl, 8.50; N, 10.09.
IR (KBr): 3035 (C-H, str., Arom), 2830(C-H, str.), 1655 (C=O, str.),624(isatin-Cl), 1530(C=C,str., Arom), 1040(C-N,str.),2832 (CH_3-Arom), 1666(C=N),3246 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.08(s,3H,-CH_3),3.06(s,1H,-CH-CO-N),2.07 (s,1H,NH), 6.70-8.66(m,09H,aromatic),4.12(s,1H,CH=C-CH_3).

6b (E)-N-(5-bromo-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carboxamide: Yield 82 %; mp 91 – 92°C; Mol. Wt: 459.28 Anal. Calcd. for$C_{23}H_{13}BrN_3O_3$ C, 60.15; H, 2.85; Br, 17.40; N, 9.15 found:C, 60.10; H, 2.82; Br, 17.34; N, 9.12. ,IR (KBr): 3030 (C-H, str., Arom), 2826(C-H, str.), 1650 (C=O, str.),620(isatin-Br), 1545(C=C,str., Arom), 1660(C=N), 1040(C-N,str.),2838 (CH_3-Arom),3224 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.7(s,3H,-CH_3),3.04(s,1H,-CH-CO-N), 4.18(s,1H,CH=C-CH_3),2.09 (s,1H,NH), 6.78-8.68(m,09H,aromatic),
6c. N(E)-N-(5-hydroxy-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]

quinoline-1(2H)-carboxamide: Yield 72 %; mp 122 – 123°C; Mol. Wt: 396.38 Anal. Calcd. for $C_{23}H_{14}N_3O_4$ C, 69.69; H, 3.56; N, 10.60 found:C, 69.65; H, 3.51; N, 10.57; IR (KBr): 3035 (C-H, str., Arom), 2854(C-H, str.), 1660 (C=O, str.),3655(isatin-OH), 1560 (C=C,str., Arom), 1052 (C-N,str.), 1680(C=N),2842 (CH_3-Arom),3236 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.9(s,3H,-CH_3),3.05(s,1H,-CH-CO-N),2.07 (s,1H,NH), 6.79-8.60 (m,09H,aromatic),2.03 (s,1H,NH), 4.14(s,1H,CH=C-CH_3).

7. The synthesis of (E)-1-((substituted-2-oxo-1l2-indolin-3-ylidene)amino)-4-methylbenzo[h]quinolin-2(1H)-one:
A (0.02 mole of each and substituted-isatin (0.02 mole) in pyridine (30 ml) was heated under reflux on a sand-bath for 6 hours. Subsequently, the reaction mixture was poured into ice-cold water (100 ml) containing conc. hydrochloric acid (10 ml). A solid started to separate out which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying in a vacuum desiccator, it was recrystallised from ethanol.

7a. (E)-1-((5-chloro-2-oxo-1l2-indolin-3-ylidene) amino)-4-methylbenzo[h]quinolin-2(1H)-one: Yield 61 %; mp 96 – 97°C; Mol. Wt: 386.82 Anal. Calcd. for $C_{22}H_{13}ClN_3O_2$ C, 68.31; H, 3.39; Cl, 9.16; N, 10.86 found:C, 68.26; H, 3.33; Cl, 9.11; N, 10.80;

IR (KBr): 3032 (C-H, str., Arom), 2842(C-H, str.), 1640 (C=O, str.),608(isatin-Cl), 1528(C=C,str., Arom), 1028(C-N,str.), 1667(C=N),2835 (CH_3-Arom),3245 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.14(s,3H,-CH_3),3.08(s,1H,-CH-CO-N),2.05 (s,1H,NH), 6.60-8.73(m,09H,aromatic), 4.01(s,1H,CH=C-CH_3).

7b.(E)-1-((5-bromo-2-oxo-1l2-indolin-3-ylidene) amino)-4-methylbenzo[h]quinolin-2(1H)-one:

Yield 59 %; mp 88 – 89°C; Mol. Wt: 431.27 Anal. Calcd. for $C_{22}H_{13}BrN_3O_2$ C, 61.27; H, 3.04; Br, 18.53; N, 9.74 found:C, 61.22; H, 3.01; Br, 18.47; N, 9.67,IR (KBr): 3028 (C-H, str., Arom), 2844(C-H, str.), 1655 (C=O, str.),620(isatin-Br), 1682(C=N), 1552(C=C,str., Arom), 1030(C-N,str.),2830 (CH_3-Arom),3238 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.07(s,3H,-CH_3),3.03(s,1H,-CH-CO-N),2.08 (s,1H,NH), 6.81-8.71(m,09H,aromatic), 4.19(s,1H,CH=C-CH_3).

7c. (E)-1-((5-hydroxy-2-oxo-1l2-indolin-3-ylidene) amino)-4-methylbenz [h]quinolin-2(1H)-one:
Yield 80 %; mp 94 – 95°C; Mol. Wt: 368.37 Anal. Calcd. for $C_{22}H_{14}N_3O_3$ C, 71.73; H, 3.83; N, 11.41 found:C, 71.65; H, 3.80; N, 11.37, IR (KBr): 3040 (C-H, str., Arom), 1682(C=N),2845(C-H, str.), 1640 (C=O, str.),3650(isatin-OH), 1545(C=C,str., Arom), 1035(C-N,str.),2840 (CH_3-Arom),3240 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.03(s,3H,-CH_3),3.08(s,1H,-CH-CO-N),2.9 (s,1H,NH), 6.84-8.63(m,09H,aromatic),2.07 (s,1H,NH),3.05(1H, s, isatin-OH), 4.10(s,1H,CH=C-CH_3).

8. The synthesis of substituted-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonothioyl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione:
A 0.02 mole of (E)-N-(substituted-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carbothioamide and thioglycollic acid containing in trace $ZnCl_2$ (0.1 gm) in DMF was heated under reflux for 4 h. It was poured into crushed ice and stirred vigorously. Solidification occurred after 15 min, which was filtered off and washed with cold water. Recrystallization from ethanol gave pure sample.

8a. 5-chloro-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonothioy) Spiro[indoline-3,5'-thiazolidine]-2,2'-dione: Yield 73 %; mp 89 – 90°C; Mol. Wt: 505.99 Anal. Calcd. For $C_{25}H_{16}ClN_3O_3S_2$ C, 59.34; H, 3.19; Cl, 7.01; N, 8.30 found: C, 59.31; H, 3.15; Cl, 7.00; N, 8.23. IR (KBr): 3046 (C-H, str., Arom),618(C-S-C), 1036 (C=S, str.), 2870 (C-H, str.), 1652 (C=O, str.),1562 (C=C,str., Arom), 1056 (C-N,str.),630 (isatin-Cl),2855 (CH_3-Arom),3242(N-H); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.1(s,3H,-CH_3),3.5 (s,1H,-CH-CO-N), 2.09(s,2H,-CH_2-spiro ring$_2$),,2.7 (s,1H,NH), 6.85-8.74 (m,9H,aromatic).

8b. 5-bromo-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonothioyl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione: Yield57 %; mp 91 – 92°C; Mol. Wt: 550.45 Anal. Calcd. for $C_{25}H_{16}BrN_3O_3S_2$ C, 54.55; H, 2.93; Br, 14.52; N, 7.63 found:C, 54.52; H, 2.88; Br, 14.47; N, 7.59, IR (KBr): 3058 (C-H, str., Arom),612 (C-S-C), 1028 (C=S, str.), 2874 (C-H, str.), 1645 (C=O, str.),1564 (C=C,str., Arom), 1065 (C-N,str.),665 (isatin-Br),2844 (CH_3-Arom),3236 (N-H); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.4(s,3H,-CH_3),3.6 (s,1H,-CH-CO-N), 2.24(s,2H,-CH_2-spiro ring$_2$),2.2 (s,1H,NH), 6.74-8.38 (m,9H,aromatic).

8c. 5-hydroxy-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonothioyl) spiro [indoline-3,5'-thiazolidine]-2,2'-dione: Yield 54 %; mp96 – 97°C; Mol. Wt: 487.55 Anal. Calcd. for $C_{25}H_{17}N_3O_4S_2$ C, 61.59; H, 3.51; N, 8.62 found: C, 61.53; H, 3.45; N, 8.56, IR (KBr): 3063 (C-H, str., Arom),620 (C-S-C), 1040 (C=S, str.), 2867 (C-H, str.), 1675 (C=O, str.),1560 (C=C,str., Arom), 1074 (C-N,str.),2865 (CH_3-Arom),3236 (N-H),3644 (isatin-OH); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.2(s,3H,-CH_3),3.5 (s,1H,-CH-CO-N), 2.24(s,2H,-CH_2-spiro ring$_2$),.2.4 (s,1H,NH),3.04 (1H, s, isatin-OH), 6.76-8.32 (m,9H,aromatic).

9. The synthesisn of substituted-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonyl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione: A 0.02 mole of (E)-N-(substituted-2-oxo-1l2-indolin-3-ylidene)-4-methyl-2-oxobenzo[h]quinoline-1(2H)-carboxamide and thioglycollic acid containing in trace $ZnCl_2$ (0.1 gm) in DMF was heated under reflux for 4 h. It was poured into crushed ice and stirred vigorously. Solidification occurred after 15 min, which was filtered off and washed with cold water. Recrystallization from ethanol gave pure sample.

9a. 5-chloro-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonyl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione:

Yield 71 %; mp 97–98°C; Mol. Wt: 489.93 Anal. Calcd. for $C_{25}H_{16}ClN_3O_4S$ C, 61.29; H, 3.29; Cl, 7.24; N, 8.58 found: C, 61.25; H, 3.24; Cl, 7.20; N, 8.53,IR (KBr): 3058 (C-H, str., Arom), 2833(C-H, str.), 1665 (C=O, str.),618(isatin-Cl), 1536(C=C,str., Arom), 1048(C-N,str.),626 (C-S-C),2830 (CH_3-Arom),3254 (N-H); Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.14(s,3H,-CH_3),3.02(s,1H,-CH-CO-N),2.04 (s,2H,-CH_2-spiro ring$_2$),2.01 (s,1H,NH), 6.76-8.62(m,09H,aromatic)

9b. 5-bromo-3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonyl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione: Yield 56 %; mp 108 – 109°C; Mol. Wt: 534.38 Anal. Calcd. for $C_{25}H_{16}BrN_3O_4S$ C, 56.19; H, 3.02; Br, 14.95; N, 7.86 found:C, 56.12; H, 3.00; Br, 14.90; N, 7.84,IR (KBr): 3033 (C-H, str., Arom), 2832(C-H, str.), 1655 (C=O, 630(C-S-C)str.),634(isatin-Br), 1552(C=C,str., Arom), 1033(C-N,str.),2845 (CH_3-Arom),3220 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.3(s,3H,-CH_3),3.2(s,1H,-CH-CO-N),2.03(s,2H,-CH_2-spiro ring$_2$),2.07 (s,1H,NH), 6.75-8.53(m,09H,aromatic)

9c. 3'-(4-methyl-2-oxo-1,2-dihydrobenzo[h]quinoline-1-carbonyl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione:
Yield 64 %; mp 106 – 107°C; Mol. Wt:471.49 Anal. Calcd. for $C_{25}H_{17}N_3O_5S$ C, 63.69; H, 3.63; N, 8.91 found:C, 63.61; H, 3.61; N, 8.86.

IR (KBr): 3048 (C-H, str., Arom),635 (C-S-C), 2862(C-H, str.), 1658 (C=O, str.),3660(isatin-OH), 1570 (C=C,str., Arom), 1064 (C-N,str.),2860 (CH_3-Arom),3242 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.6(s,3H,-CH_3),3.08 (s,1H,-CH-CO-

N),2.10 (s,2H,-CH_2-spiro ring),2.6 (s,1H,NH), 6.72-8.44 (m,09H,aromatic),2.09 (s,1H,NH),3.12(1H, s, isatin-OH)

10. The synthesis of substitutes-3'-(4-methyl-2-oxobenzo[h]quinolin-1(2H)-yl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione:

A 0.02 mole of each (E)-1-((substituted-2-oxo-1l2-indolin-3-ylidene) amino)-4-methylbenzo[h]quinolin-2(1H)-one and thioglycollic acid containing in trace $ZnCl_2$ (0.1 gm) in DMF was heated under reflux for 4 h. It was poured into crushed ice and stirred vigorously. Solidification occurred after 15 min, which was filtered off and washed with cold water. Recrystallization from ethanol gave pure sample.

10a. 5-chloro-3'-(4-methyl-2-oxobenzo[h]quinolin-1(2H)-yl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione:

Yield 59 %; mp 111 – 112°C; Mol. Wt: 461.92 Anal. Calcd. for $C_{24}H_{16}ClN_3O_3S$ C, 62.41; H, 3.49; Cl, 7.67; N, 9.10 found: C, 62.37; H, 3.46; Cl, 7.62; N, 9.07.
IR (KBr): 3042 (C-H, str., Arom),625(C-S-C), 2846(C-H, str.), 1654 (C=O, str.),625(isatin-Cl), 1540(C=C,str., Arom), 1036(C-N,str.),2830 (CH_3-Arom),3242 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.11(s,3H,-CH_3),3.09(s,1H,-CH-CO-N),2.09 (s,2H,-CH_2-spiro ring$_2$),2.02 (s,1H,NH), 6.73-8.58(m,09H,aromatic)

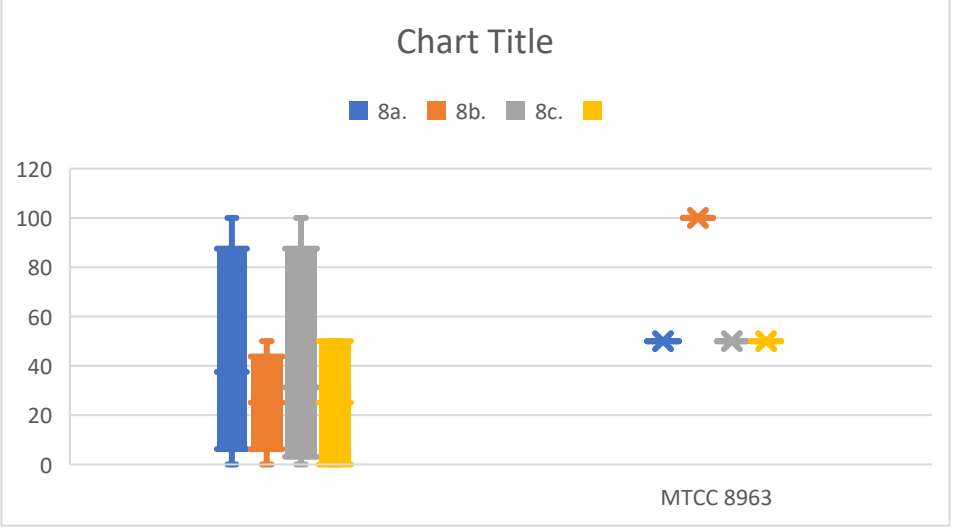
10b. 5-bromo-3'-(4-methyl-2-oxobenzo[h]quinolin-1(2H)-yl) spiro[indoline-3,5'-thiazolidine]-2,2'-dione:
Yield 56 %; mp 93 – 94°C; Mol. Wt: 506.37 Anal. Calcd. for $C_{24}H_{16}BrN_3O_3S$ C, 56.93; H, 3.18; Br, 15.78; N, 8.30 found:C, 56.90; H, 3.13; Br, 15.72; N, 8.27,IR (KBr): 3048 (C-H, str., Arom),628(C-S-C), 2838(C-H, str.), 1664 (C=O, str.),640(isatin-Br), 1560(C=C,str., Arom), 1042(C-N,str.),2830 (CH_3-Arom),3228 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.09(s,3H,-CH_3),3.07(s,1H,-CH-CO-N),2.08 (s,2H,-CH_2-spiro ring$_2$),2.06 (s,1H,NH), 6.85-8.62(m,09H,aromatic)
10c. 5-hydroxy-3'-(4-methyl-2-oxobenzo[h]quinolin-1(2H)-yl)spiro[indoline-3,5'-thiazolidine]-2,2'-dione: Yield 61 %; mp 116 – 117°C; Mol. Wt: 443.48 Anal. Calcd. for $C_{24}H_{17}N_3O_4S$ C, 65.00; H, 3.86; N, 9.48 found: C, 64.97; H, 3.80; N, 9.43, IR (KBr): 3034 (C-H, str., Arom),616(C-S-C), 2862(C-H, str.), 1652 (C=O, str.),3660(isatin-OH), 1565(C=C,str., Arom), 1056(C-N,str.),2840 (CH_3-Arom),3235 (N-H),; Hydrogen-1 nuclear magnetic resonance-$CDCl_3$-300 MHz δ in ppm : 1.7(s,3H,-CH_3),3.5(s,1H,-CH-CO-N),2.14 (s,2H,-CH_2-spiro ring$_2$),2.9 (s,1H,NH), 6.72-8.57(m,09H,aromatic),3.09(1H, s, isatin-OH)

TABLE- I: In vitro anti-microbial activity 4-Methyl-2H-benzo[h]chromen-2-one based Spiro derivatives MIC (mg/ml) of compounds 8a-c,9a-c and 10a-c:

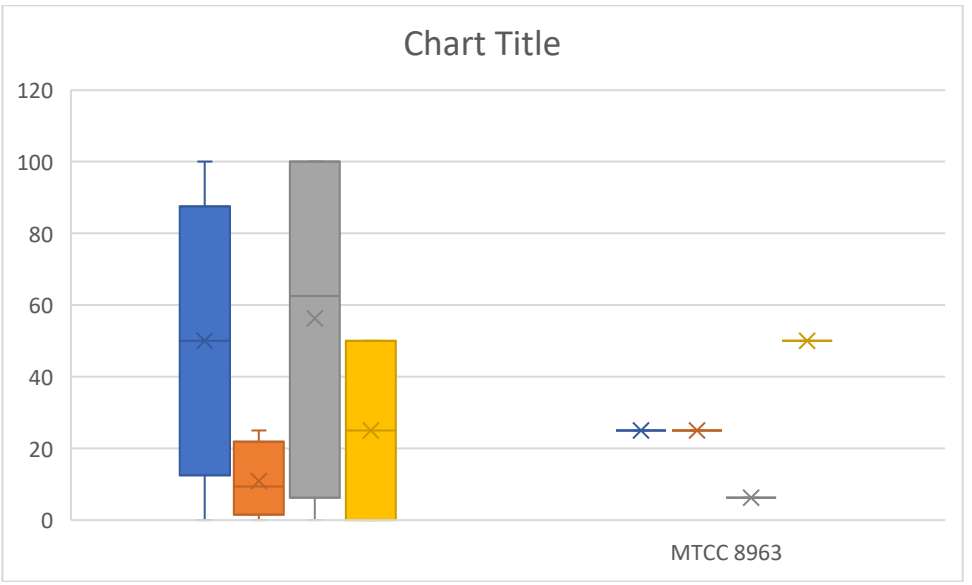
S.No.	R-Isatin	Gram -ve	Gram +ve

		Escherichia coli MTCC 443	Salmonella typhi ATCC 6539	Corynebacterium striatum MTCC 8963	Staphylococcus pyogenes MTCC 442
8a.	5-chloroisatin	100	25	50	50
8b.	5-bromoisatin	50	25	100	25
8c.	Isatin	12.5	100	50	50
9a.	5-chloroisatin	50	50	25	100
9b.	5-bromoisatin	25	6.25	25	12.5
9c.	Isatin	25	100	6.25	100
10a.	5-chloroisatin	100	50	50	100
10b.	5-bromoisatin	50	12.5	50	25
10c.	Isatin	50	50	100	25
	Ciprofloxacin		50	50	

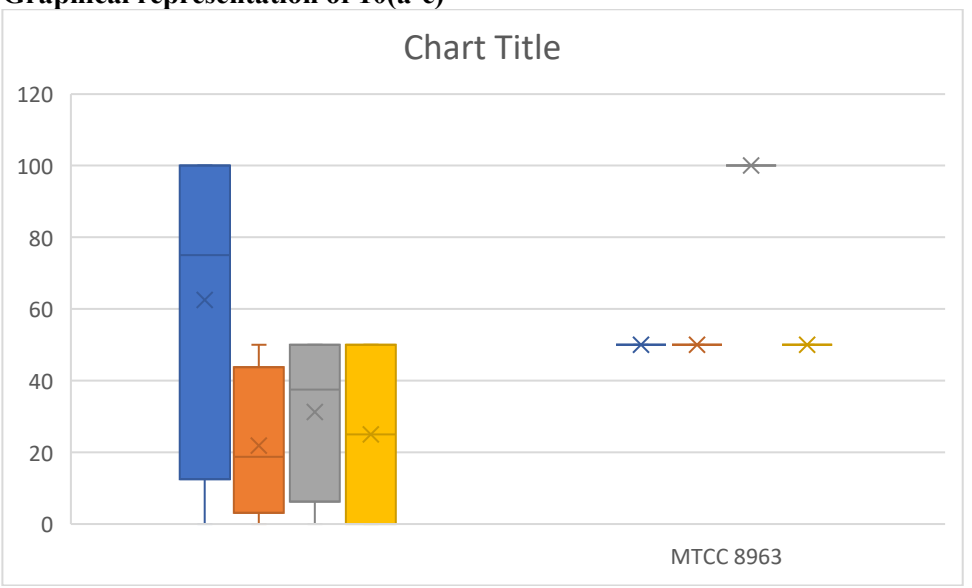
Graphical representation of 8(a-c)



Graphical representation of 9(a-c)



Graphical representation of 10(a-c)



In vitro anti-bacterial susceptibility test (AST):

The -4-Methyl-2H-benzo[h]chromen-2-one based Spiro heterocyclic derivatives (8a-c,9a-c and 10a-c) were tested in vitro for their anti-bacterial against Gram -ve and Gram +ve species Escherichia coli, Salmonella typhi, Corynebacterium striatum and Staphylococcus pyogenes.

The minimum inhibitory concentration (MIC) and in vitro antbactrial activity of the tested derivatives were checked using the Borth dilution method, which is shown in table I. All prepared samples were incubated at 37°C for 24 hours for antbactrial screening. The absorbance at 600 nm was used to calculate the growth of bacteria in each conical tube. Each conical tube holding the test chemical at concentrations of 1000, 500, 250, 125, 62.5, and as on μg ml−1 in 10 ml of LB media was filled with 100 μl of the microbial suspension for this purpose. By concurrently adding 100 μl of the microbial suspension to a conical tube that contained 10 ml of LB media, a similar experiment was carried out using Ciprofloxacin as the control. For every conical tube, the optical density of the fluid was recorded at 600 nm after 24 hours to determine the proliferation of all bacterial gram -ev and Gram +ev species. Graph of optical density against compound concentration were made. The MIC of a chemical was determined by measuring the drop in the OD of microorganisms at that concentration.

Table I shown the outcomes of the antbactrial activity. Few derivatives displayed a minimum inhibitory concentration (MIC) value that was comparable to that of the standard medications, while the remaining derivatives displayed MIC values that were either low or high. activity 4-Methyl-2 H-benzo[h]chromen-2-one based Spiro derivatives 9b and 9c found higest active against Salmonella typhi ATCC 6539 and Corynebacterium striatum MTCC 8963 at 6.25 μg $ml^{-1.}$ with R=Br-isatin and H-isatin are present on phenyl ring

The secone lowest MIC value and most valuable compounds exhibit by 9b and 10b 12.5μg ml^{-1} against Staphylococcus pyogenes MTCC 442 and Salmonella typhi again with 5-bromoisatin.

Although 9 derivatives 8a,8b against Salmonella typhi, 8b- Staphylococcus pyogenes MTCC 442, 9a,9b- Corynebacterium striatum MTCC 8963,9b,9c- Escherichia coli MTCC 443 and 10b,10c-- Staphylococcus pyogenes MTCC 442 showed good activity

Rest all derivatives with R-isatin = 5-Chloro,5- Bromo and H give comparatively good antibacterial activity.

Result AND Discussion:

Antibactrial activity: In Gram positive bacteria strains, Oxazin Dearvatives 9b and 9c showed activity against Staphylococcus pyogenes and Corynebacterium where as In Gram negative 8c and 9b, 10b Escherichia coliand Salmonella typhi and showed very good activity (6.25-50μg/mL) compared with Ciprofloxacin. All other compounds show moderate activity or less activity against all microbes (Table 1).

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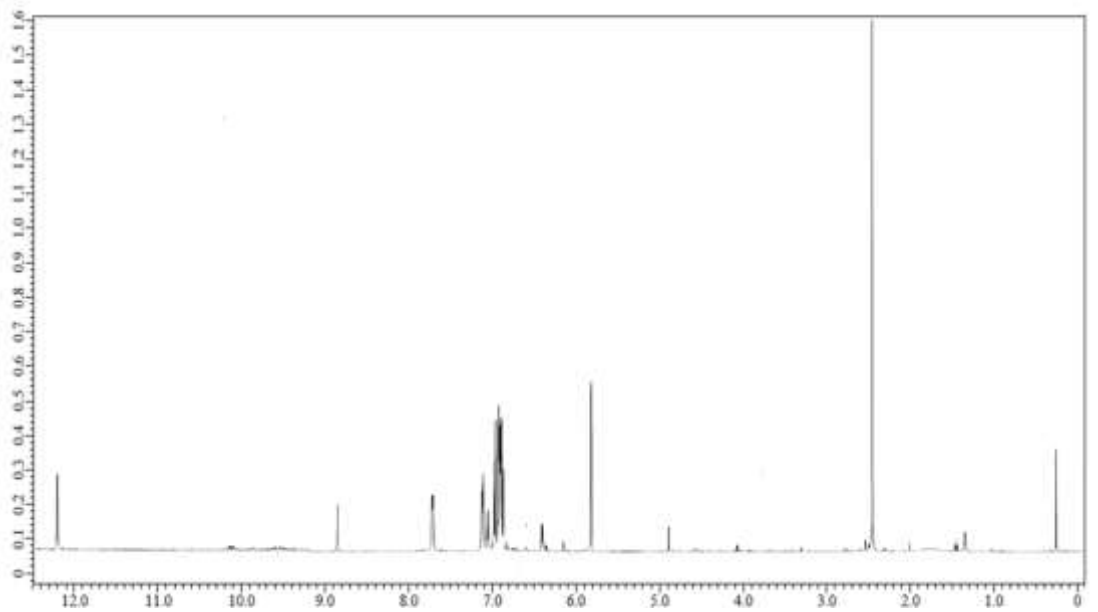

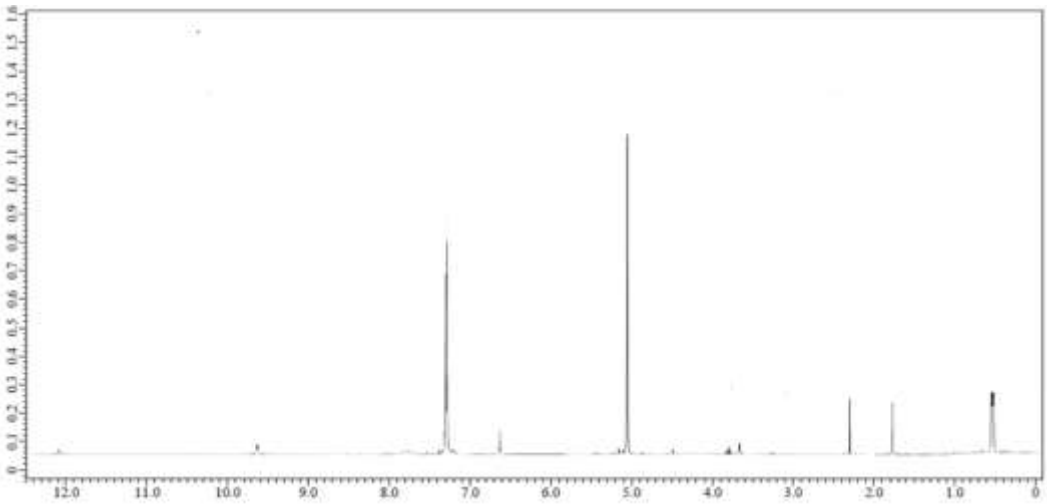

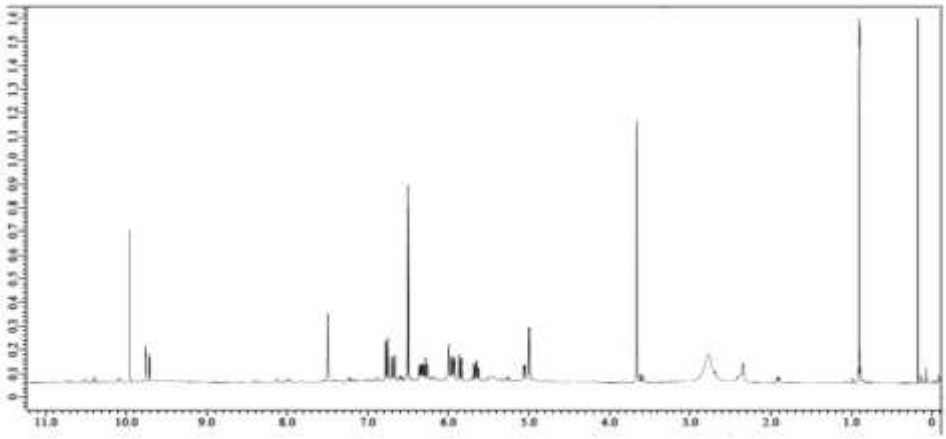

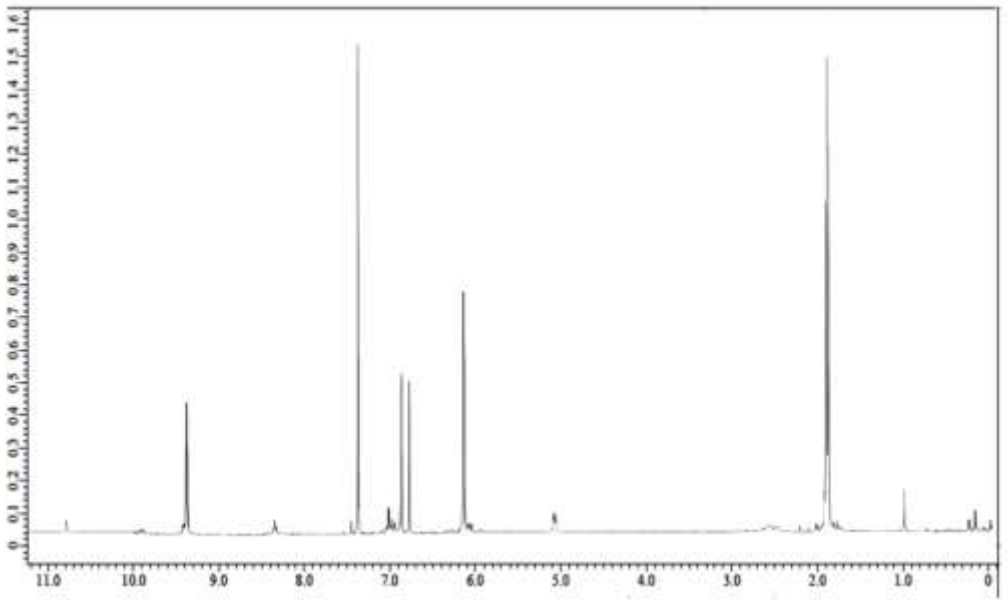
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