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SYNTHESIS AND EVALUATION OF NOVEL BENZOXAZINONE DERIVATIVES

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Abstract : Medicinal chemists were drawn to investigate lead compounds for the therapy of many microbial illnesses due to the benzoxazinone scaffold's versatile biological activity profile. The unquestionable antibacterial qualities of thiosemicarbazones were confirmed in extensive literature, which prompted our current investigation. Condensation of substituted anthranilic acid (1a1c) with acetyl chloride (2) yields methyl bzoxazine-4-ones (3a-3c), which are oxidized with selenium dioxide to the corresponding aldehydes (4a-4c), and then condensation with different thiosemicarbazides to create novel benzoxazinonethiosemicarbazone (5a-5c) hybrids. The synthesis of fifteen new benzoxazinone-thiosemicarbazones (5a1-5a5, 5b1-5b5, and 5c1-5c5) in sufficient quantities was followed by a thorough spectral investigation of the compounds utilizing sophisticated analytical tools. The compounds with the names were tested for antifungal and antibacterial properties. According to the results, every synthetic molecule was shown antibacterial qualities. It was argued that compound 5b4 possessed strong antibacterial qualities against the specified types of bacteria and fungi.

Index Terms: Benzoxazinone, Thiosemicarbazides, Thiosemicarbazone, Antibacterial, Antifungal, Agar disc diffusion

1. INTRODUCTION

Heterocycles are important pharmacophores and have significance to create privileged chemical structures possessing pharmacological activities. Benzoxazinones motifs are the key backbone of numerous biological active molecules and make a prevalent impact on thefield of medicinal chemistry. Benzoxazine moiety is present in several structures endowed with various activities, such as antiallergics antimicrobials , anti-mycobacterials

Materials and Methods

Melting points are uncorrected and were determined using sulphuric acid bath in open glass capillaries. IR spectra were measured on a Perkin-Elmer 1430 Infrared spectrophotometer using KBr discs. NMR (1H and 13C) spectra were recorded on a Bruker spectrophotometer at 400 MHz in DMSO-d6 solvent using tetramethyl silane (TMS) as an internal standard and chemical shift (δ) are reported in ppm. Mass spectra were recorded on a Finnigan model SSQ/7000 mass spectrometer (70ev). All the reactions were monitored by thin-layer chromatography (TLC) on silica gel (60 GF 353; Merck) followed by iodine vapour and UV light visualization techniques. All chemicals, and solvents, were purchased from Sigma–Aldrich. The scheme of synthesis for the novel benzoxazinone thiosemicarbazone hybrids was depicted in Figure

Synthesis of Benzoxazinone thiosemicarbazone derivatives

General procedure for the synthesis of substituted benzoxazinones (3a-3c)

An 100ml round bottomed flask was charged with 0.01moles of substituted anthranilic acid (1a,1b,1c) and pyridine (20ml). To this solution 0.02moles of acetyl chloride was carefully added and the RBF kept in an ice bath at -5^{0}C as the reaction is exothermic in nature. Then the reaction mixture was stirred at 0^{0}C for 10mins and after contents of RBF were slowly allowed to stir under room temperature until the completion of the reaction. Reaction progress was monitored by TLC with Ethyl acetate: Hexane (10:90 v/v) mobile phase. "The reaction mixture was poured into ice cold water (200 mL) and precipitates were filtered off. The residue was washed with cold water (3 × 40 mL) and dried. Substituted benzoxazin-4-ones were crystallized from ethanol".

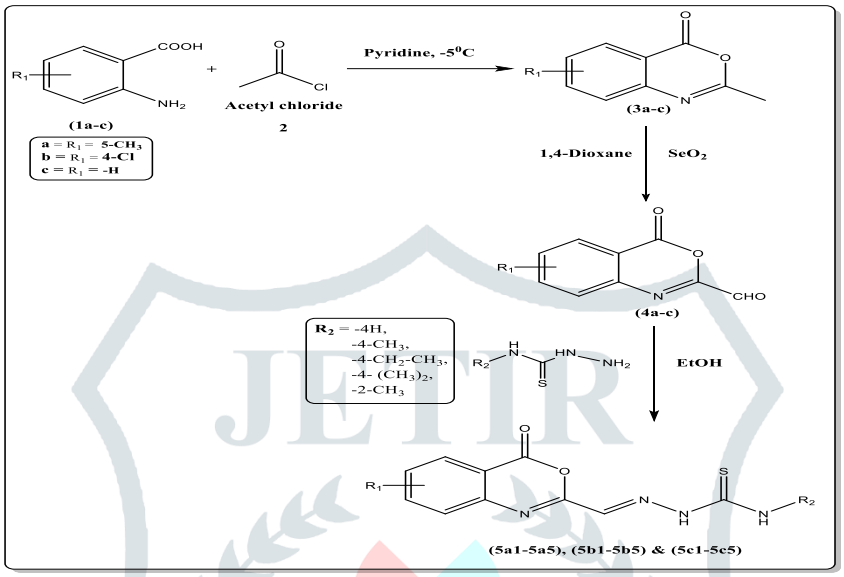


Figure. Scheme of synthesis of novel benzoxazinone-thiosemicarbazone hybrids

General procedure for the synthesis of substituted benzoxazinone carbaldehydes (4a-4c)

In a 100ml RBF containing 50ml of 1,4-dioxane, substituted benzoxazin-4-ones (3a-3c) (10 mmol, 1equv) and selenium dioxide (20 mmol, 2 equv) were added, then refluxed for 2hrs. Reaction mass was allowed to cool to room temperature. A small amount of precipitated formed in the room temperature then it was filtered off and the filtrate pH adjusted to 7.0 with 5% sodium bicarbonate solution. "The separated solid was removed by filtration, and the filtrate was extracted four times with dichloromethane (15 mL, each) and combined, washed with brine then dried over anhydrous sodium sulphate overnight. The solvent was removed by rotary evaporation, and the residue was re-crystallized from methanol to produce substituted benzoxazinone carbaldehydes"**(4a-4c).**

General procedure for the synthesis of Benzoxazinone-thiosemicarbazone derivatives (5a1-5a5, 5b1-5b5 &5c1-5c5)

New thiosemicarbazone hybrids (5a1-5, 5b1-5 and 5c1-5) were prepared by condensation of thiosemicarbazide, 4-methylthiosemicarbazide, 4-ethylthiosemicarbazide, 4-dimethylthiosemicarbazide,and 2-methylthiosemicarbazide, with corresponding aldehyde derivatives **(4a-4c).**

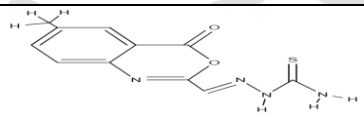
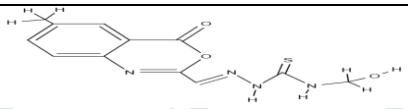
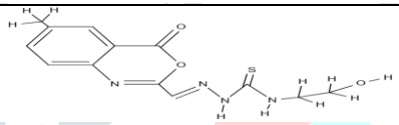
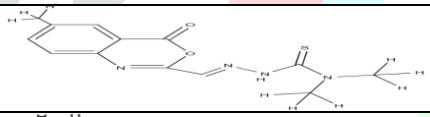
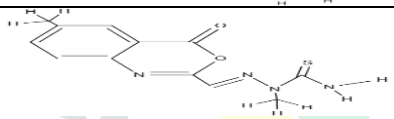
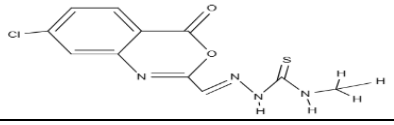
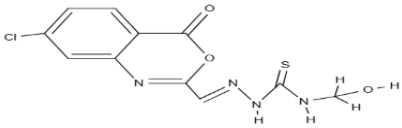
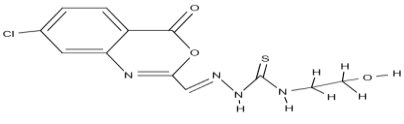
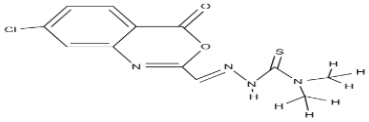
"A solution of 4a, 4b and 4c (5.0 mmol) in ethanol (20 ml) were separately added to a solution of different thiosemicarbazides (5.0 m mol) in ethanol (20 ml). The resulting yellow solution was refluxed with stirring for 2 h. White precipitates appeared when the solution cooled down to room temperature, then filtered and washed with fresh ethanol. The resulting material was re crystalized and air-dried to get the final products **5a1-5a5, 5b1-5b5 & 5c1-5c5.** Each of the products so obtained was washed with the distilled and cooled ethanol to remove the un reacted material

Biological Evaluation

In vitro antibacterial assay

All the bacterial strains used in this experiment were obtained from the department of microbiology, Osmania University and preserved at 4ºC. Antimicrobial activity of the prepared hybrids **(5a1-5a5, 5b1-5b5 & 5c1-5c5)** was evaluated using disc-diffusion method against gram positive bacteria (Bacillus subtilis, and Staphylococcus aureus*)* and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa). "Ampicillin (100µg/ml) in DMSO was used as reference antibiotics. Nutrient agar medium was taken in the pre-sterilized petri-dishes and the microorganisms were grown by inoculating 0.5 ml of spore suspension (108 spores/ml) culture broth. A stock solution for all the prepared compounds **(5a1-5a5, 5b1-5b5 & 5c1-5c5)** was made by using DMSO. The disc (6 mm in diameter) was stuffed with 200 µg/ml, 100 µg/ml and 50 µg/ml of each test solution, placed on the seeded Nutrient agar medium and the petri-dishes were incubated at 37˚C for 24 hr. DMF alone was used as control at the equal preceding concentration. Zone of inhibition of each compound was recorded in mm". The experiment was done in triplicates

Synthesis results Table 1 : Molecular formula, melting point and yield of compounds

prod. No	Ṙ1	Ṙ2	Structure	Molecular formula	Melting point °C	% Yield
5.à$_1$	-CH_3	-H		$C_{11}H_{10}N_4O_2S$	213-214	73.6
5. à$_2$	-CH_3	-CH_2OH		$C_{12}H_{13}N_4O_3S$	202-231	86.50
5. à$_3$	-CH_3	-C_2H_5OH		$C_{13}H_{16}N_4O_3S$	219-226	89.40
5. à$_4$	-CH_3	-$(CH_3)_2$		$C_{13}H_{14}N_4O_2S$	219-220	79
5. à$_5$	-CH_3	-CH_3		$C_{12}H_{12}N_4O_2S$	211-212	80
5.b$_1$	-Cl	-CH3		$C_{12}H_{12}ClN_4O_2S$	227-228	78
5. **b** $_2$	-Cl	-CH_2OH		$C_{12}H_{12}ClN_4O_3S$	233-236	95
5. b$_3$	-Cl	-C_2H_4OH		$C_{13}H_{14}ClN_4O_3S$	241-242	96
5.b$_4$	-Cl	-$(CH_3)_2$		$C_{13}H_{13}ClN_4O_2S$	234-235	78

RESULTS AND DISCUSSION

Chemistry

In this study, novel thiosemicarbazone derivatives containing the benzxoxazinone moiety were prepared according to reaction as in Scheme 1. The condensation reaction between commercially available substituted anthranilic acid (1a–1c) with acetyl chloride (2) in the presence of pyridine yielded good amounts of substituted 2-methyl benzoxazinone intermediate (3a-3c). The intermediate 3a-3c, were oxidized to their aldehyde derivatives (4a-4c) by the reaction with selenium dioxide. Further, various substituted aryl thiosemicarbazides were added to ethanolic solutions of benzoxazinone carbaldehydes (3a–3c) to get a series of corresponding benzoxazinone-thiosemicarbazone derivatives (5a1-5a5, 5b1-5b5 & 5c1-5c5).

ANALYTICAL DATA

The structural confirmation of the IR, MASS, and NMR spectra recordings have been used to create molecules that are synthetic

Spectral data of the chemicals were enumerated here:

According to the plan outlined above in the Figure 4.11, fifteen derivatives were synthesized, yield was calculated and structures were established. The structures and physical details were depicted in the Table 3.1, and the IR, H^1NMR and MASS spectral data reported below:

(e)-2-((6.-methyl- four-oxo-4.H-benzo[d.], [0ne, three.] oxazine-two-yl) methylene) Hydrazine-1-carbothioamide

IR. (KBr.), light yellowish crystalline material: ν_{max} in cm^{-1}: 1589.12 (Carbon =Nitrogen), 3186.1 (Nitrogen - Hydrogen), 3141.3 (= Carbon –Hydrogen), 1311.3 (Carbon –Nitrogen),1492.8 (Carbon = Carbon), 1116.9 (Carbon =Sulfur); **^{1}H N.M.R** (350 -450. M. Hz, D.M.S.0-*d*.6) 1H N.M.R: δ 2.18 (3H, s), 6.39 (1H, dd, Joules, 7.99, 2.5 Hz), 6.51 (1 hydrogen, *d d* Joules = 6.32, 1.5 Hz), 7.63 (1 hydrogen, *d*, Joules 2.3, 1.49 Hz), 6.49 (1Hydrogen, s). **E. S. I-M.S:** mass/molecular weight Analysis. Estimated as compound ($[M + H]^+$): 254.18, observed 223.41.

Hydrazine-1-carbothioamide (e)-N-alcohol -two-((6-methyl-4-oxo-4H-benzo[one, three] oxazin-two-yl) methylene)

IR (KBr), light yellowish crystalline material: ν_{max} in cm^{-1}: 1731 (Carbon =Nitrogen), 2934.3 (Nitrogen - Hydrogen), 3427.1 (=Carbon –Hydrogen), 1352.8 (Carbon –Nitrogen),1439.4 (Carbon = Carbon), 1148.1 (Carbon =Sulfur); **^{1}H NMR** (550 Hz D.M.S.O-*d*.7) δ 12.37 (s, 1H), 7.20 (q, Joules. 5 H.z, 1 hydrogen), 6.82 – 6.47 (m, 1 hydrogen), 6.43 (s, 1 hydrogen), 6.11 (Joules = 1.2 Hz, 2 hydrogen), 2.097 (s, 3H), 1.98 (s, 3H). **E.S.I-M. S:** m/z Analysis. Estimated as compound ($[M + H]^+$): 284.21, observed 282.16.

Hydrazine-1-carbothioamide (e)-N-ethylalcohol- two- (six-carbyl-4-oxo-4H-benzo[1,4] oxazin-3-yl) methylene)

IR (KBr), pale yellow crystalline material. **):** ν_{max} in cm^{-1}: 15098.1 (Carbon =Nitrogen), 2915.5 (Nitrogen - Hydrogen), 2871.9 (=Carbon –Hydrogen), 1189.7 (Carbon –Nitrogen),1391.7 (Carbon = Carbon), 1131.1 (Carbon =Sulfur); **^{1}H N.M.R** (450 – 550 Hz, D.M.S.0-*d*six) δ 10.12 (s, 1Hydrogen), 8.12 (Joules 3.1.0 Hz, 1Hydrogen), 7.13 – 7.89 (1Hydrogen), 7.34 (1 hydrogen), 8.01 (d, Joules = 01.21 Hz, 2 hydrogen), 03.45 (Joules 8.7, 3.29 Hz, 2Hydrogen), 2.14 (s, 3 hydrogen), 03.13 (Joules = 7.4 Hz, 3Hydrogen). **E.S.I-M.S:** m/z Anal. Calcd. For compound ($[M + H]^+$): 279.43, observed 287.13.

Hydrazine-1-carbothioamide (e)-N, N, 2-methyl-2-((6-methyl-4-oxy-4Hydrogen-benzol [one, three] oxazin-2-yl) methyleneoxide

IR (KBr), pale yellow crystalline material **:** ν_{max} in cm^{-1}: 1524.3 (Carbon =Nitrogen), 2927.4 (Nitrogen - Hydrogen), 2919.2 (=Carbon –Hydrogen), 1141.3 (Carbon –Nitrogen),1425.1 (Carbon = Carbon), 1124.5 (Carbon =Sulfur); **^{1}H N. M .R** (450 – 500 Hz, D.M.S.0-*d*.5)11.13 (s, 1Hydrogen), 4.11 – 5.04 (m, 1Hydrogen), 5.19 (s, 1Hydrogen), 4.19 (Joules = 2.4 Hz, 2Hydrogen), 1.25 (s, 6 hydrogen), 3.93 (s, 3Hydrogen). **E.S.I-M.S:** mass/ atomic number Analysis. Estimated as ($[M + H]^+$): 272.41 observed 288.43.

One carbyl -2- ((six-mcarbyl-4-oxo-4hydro-benzo[one ,three] oxazin-2-yl) methylene) hydrazine-1-carbothioamide (5.a.5)

IR (KBr), pale yellow crystalline material **:** ν_{max} in cm^{-1}: 1513.3 (C=N), 2817.1 (Nitrogen - Hydrogen), 2863.2 (=Carbon –Hydrogen), 1288.5 (C-N),1414.1 (Carbon = Carbon), 1013.3 (Carbon =Sulfur); **^{1}H N..MR** (450 to 550 Hz, D.M.S.0-d.6) δ 08.44 (s, 2 hydrogen), 6.890(q, Joules 0.91 Hz, 1Hydrogen, 6.93 (*d*, J = 01.29 Hz, 2 hydrogen), 7.021 (s, Hydrogen), 3.34 (s, 3Hydrogen), 1.98 (s, 3 hydrogen). **E.S.I-M.S:** mass/molecular number Analysis. Estimated as compound ([M + hydrogen] $^+$): 293.81, observed 298. 23.

(5.b.1) oxazin-2-yl)methylene)hydrazine-1-carbothioamide (e)-2- ((7-Cl-4-oxo-4H-benzo[1,3]

IR (KBr), pale yellow crystalline material **:** ν_{max} in cm^{-1}: 1612.1 (Carbon =Nitrogen), 3012.3 (Nitrogen - Hydrogen), 2945.1 (=Carbon –Hydrogen), 1194.3 (Carbon –Nitrogen),1421.3 (Carbon = Carbon), 1131.6(Carbon =Sulfur); **^{1}H NMR** (600 MHz, D.M.S.O-d.6) δ 12.23 (s, 1Hydrogen), 10.13 (s, 2 Hydrogen), 09.40 – 09.25 (m, 1 hydrogen), 8.42 – 8.91 (m, 2 hydrogen), 6.99 (s, 1 hydrogen). **E.S.I-M.S:** m/z Analysis. Estimated as compound ([M + Hydrogen]$^+$): 261.42 observed 263.78.

N-methylhydrazine-1-carbothioamide, (e)-two-(7-Cl-4-oxo-4Hydro-benzo [one, three] oxazin-2-yl) methylene),

IR (KBr), pale yellow crystalline material **:** ν_{max} in cm^{-1}: 1572.4 (Carbon =Nitrogen), 3134.1 (Nitrogen - Hydrogen), 2947.1 (=Carbon –Hydrogen), 1139.8 (Carbon –Nitrogen),1419.1 (Carbon = Carbon), 1101.6 (Carbon =Sulfur); **^{1}H NMR** (600 MHz, D.M.S.O-delta.6) delta 10.91 (s, 1Hydrogen), 7.21 – 7.13 (2 hydrogen), 6.91 – 7.03 (2 hydrogen), 6.89 (s, 1H), 03.07 (s, 3 hydrogen). **E.S.I-M.S:** m/z Analysis. Estimated as compound (M + H]$^+$): 301.21, observed 312.31.

N-ethylhydrazine-1-carbothioamide (e)-two-((7-Cl-four -oxo-4Hydro-benzo[one ,three] oxazin-2-yl) methylene

IR (KBr), pale yellow crystalline material **:** ν_{max} in cm^{-1}: 1689.5(Carbon =Nitrogen), 3324.2(N-H), 2843.1 (=Carbon –Hydrogen), 1139.4 (Carbon –Nitrogen),1491.9 (Carbon = Carbon), 1075.5 (Carbon =Sulfur); **^{1}H NMR** (600 MHz, D.M.S.0-d.6) δ 10.12 (s, 1H), 9.29 – 9.31 (1Hydrogen), 8.12 (Joules 4.10 Hz, 1Hydrogen), 8.41 – 8.92 (m, 2Hydrogen), 8.41 (s, 1 Hydrogen), 4.21 (Joules = 7.3, 1.2 Hz, 2 Hydrogen), 2.10 (Joules = 5.4 MHz, 3H). **E.S.I-M.S:** m/z Analysis. Estimated as compound ([M + H]$^+$): 323.13, observed 303.78.

Oxazin-2-yl) methylene (e)-para -((7-chloro-4-oxo-4H-benzo[d] [1,3])-N, N-dimethylhydrazine-1-carbothioamide (5b4)

IR (KBr), light yellow crystalline material ν_{max} in cm^{-1}: 1583.1 (Carbon =Nitrogen), 3901.4 (Nitrogen - Hydrogen), 2971.3 (=Carbon –Hydrogen), 1131.7 (Carbon –Nitrogen),1412.9 (Carbon = Carbon), 1173.1 (Carbon =Sulfur); **^{1}H NMR** (600 Hz, D.M.S.O-*d*.5) δ 10.14 (Hydrogen), 10.08 – 10.13 (mass, 1Hydrogen), 7.12 – 7.23 (mass.2 hydrogen), 8.19 (1 hydrogen), 4.11 (s, 6 hydrogen). **E.S.I-M. S:** mass/molecular weight Analysis. Estimated as compound ([M + H]$^+$): 312.89, observed 314.10.

(e)-two-(seven-Cl-4-oxy-4 Hydro. -benzo [one, three] oxazine-2-yl) methanediyl)-1-methoxylhydrazine-one -carbothioamide

IR (KBr), Light yellow crystalline material ν_{max} in cm^{-1}: 1579.02 (Carbon =Nitrogen), 3015.2 (Nitrogen - Hydrogen), 2731.3 (=Carbon –Hydrogen), 1134.1 (Carbon –Nitrogen),1494.8 (Carbon = Carbon), 1156.6 (Carbon =Sulfur); **^{1}H NMR** (600 MHz, D.M.S.0-*d*.6) delta 8.14 (s, 2Hydrogen), 10.01 – 10.15 (1Hydrogen), 9.19 – 9.10, 2 hydrogen, 7.34. (s 1H), 4.59,3 hydrogen. **E.S.I-M.S:** m/z Analysis. Estimated as compound ([M + H]$^+$): 321.40, observed 329.30.

(e)-2-(four -oxy-4Hydo-benzo[one,three] oxazine-2-yl) methanediyl) hydrazin-1-carbothioamide.

IR (KBr), light yellow crystalline material ν_{max} in cm^{-1}: 1512.2 (Carbon =Nitrogen), 3141.3 (Nitrogen - Hydrogen), 3193.9 (=Carbon –Hydrogen), 1301.9 (Carbon –Nitrogen),1497.9 (Carbon = Carbon), 1158.9 (C=S); **^{1}H NMR** (600 MHz, D.M.S.O-delta.6.0) delta 12.91 (1 Hydrogen), 12.51,2 hydrogen, 9.15 (dd, J = 6.07, 1.9 Hz, 1 Hydrogen), 9.45 – 9.29 (m, 3 hydrogen), 9.17 (s, 1Hydrogen). **E.S.I-M.S:** m/z Analysis. Estimated as ([M + Hydrogen] $^+$): 267.12, observed 272.91.

(e)-N-alkyl-2-(four -oxy-4Hydrogen -benzo[one, three] oxazine-2-yl) methanediyl) hydrazine -one -carbothioamide (5.c.2)

IR (KBr), pale yellow crystalline material ν_{max} in cm^{-1}: 1569.2 (C=N), 3141.2 (Nitrogen - Hydrogen), 3131.4 (=Carbon –Hydrogen), 1181.2 (Carbon –Nitrogen),1461.4 (Carbon = Carbon), 1151.6(C=S); **^{1}H*ydrogen* N.M.R** (550 Hz, D.M.S.0-delta.7) delta 11.23 (1Hydrogen), 10.24 (Joules = 3.0 mHz,,Hydrogen), 11.12 (Joules,6.11, 1.13 mHz,Hydrogen), 8.15 – 8.11 (2 Hydrogen), 8.62 – 8.13 (Hydrogen), 8.13 (s, 1Hydrogen), 4.13 (1 Hydrogen). **E.S.I-M.S:** mass/molecular weight. Analysis. Estimated as ($[M + H]^+$): 283.43, observed 287.21.

A compound derived from hydrazine-1-carbothioamide and N-ethyl-substituted benzo[d]oxazine, featuring a methylene linkage at the 2-position and a ketone functional group at the 4-position of the oxazine ring **(5.c.3)**

IR (KBr), pale yellow crystalline material ν_{max} in cm^{-1}: 15079.0 (Carbon =Nitrogen), 3091.8 (Nitrogen - Hydrogen), 2941.1 (=Carbon –Hydrogen), 1109.12 (Carbon –Nitrogen), 1492.0 (Carbon = Carbon), 1131.3t (C=S); **^{1}H N. M. R** (600 Hz, D.M.S.0-delat.7) delta 12.14 (s,Hydrogen), 10.23 (Joules,10.21, 2.8 Hz,hydrogen), 10.11 (Joules 3.0 Hz, 1 hydrogen), 11.34 – 11.24 (3Hydrogen), 12.04 (s, Hydrogen), 5.12 (Joules,7.2, 1.8 Hz, 2Hydrogen), 1.25 (Joules, 7.9 Hz, 3 hydrogen). **E.S.I-M.S:** m/z Analysis. Estimated as compoumd ($[M + H]^+$): 307.13, observed 312.19.

Oxazin-2-yl) methylene) hydrazine-1-carbothioamide (e)-N, N- methoxymethane -2-((4-oxo-4H-benzo[one,three]) (5.c.4)

IR (KBr), pale yellow crystalline material ν_{max} in cm^{-1}: 1571.7 (Carbon =Nitrogen), 3031.5 (Nitrogen - Hydrogen), 3292.2 (=Carbon –Hydrogen), 1137.2 (Carbon –Nitrogen),1642.9 (Carbon = Carbon), 1134.1 (Carbon =Sulfur); **^{1}H N.M.R** (600 MHz, D.M.S.O-d.6) δ 12.35 (s, 1Hydrogen), 7.85 (dd, J = 6.0, 01.8 Hz, hydrogen), 10.12 – 10.43 (m, 2Hydrogen), 07.34 (1hydrogen), 6.23 (s, 4Hydrogen). **E.S.I-M. S:** mass/molecular weight, estimated as compound ($[M + H]^+$): 213.31, observed 217.10.

Hydrazine-1-carbothioamide (e)-1-alkyl-two-(6-methyl-4-oxy-4hydro-benzo-1,3-oxazin-2-yl) methylene) (5.c.5)

IR (KBr), pale yellow crystalline material **:** ν_{max} in cm^{-1}: 1613.7 (Carbon =Nitrogen), 3013.1 (Nitrogen - Hydrogen), 2965.1 (Carbon –Hydrogen), 1142.7 (Carbon –Nitrogen), 1414.2 (Carbon = Carbon), 1104.9 (Carbon =Sulfur); **^{1}H NMR** (600 MHz, D.M.S.O-delta.7) delta 10.11 (s, 2Hydrogen), 10.23 – 10.56 (Hydrogen), 10.23 (Joules,1.2 Hz, 2Hydrogen), 10.12 (s, 1Hydrogen), 3.10 (s, 1Hydrogen), 3.13 (2Hydrogen). **E.S.I-M.S:** mass/ molecular weight Estimated as $C_{12}H_{12}N_4O_2S$ ($[M + H]^+$): 2872.22, observed 292.05.

Spectral data for compound 5a1

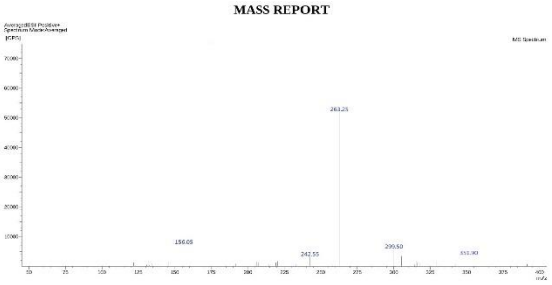


Fig. 4.1: Spectral data 5a1

Fig. 4.2: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.a.1

Spectral data for compound 5a2

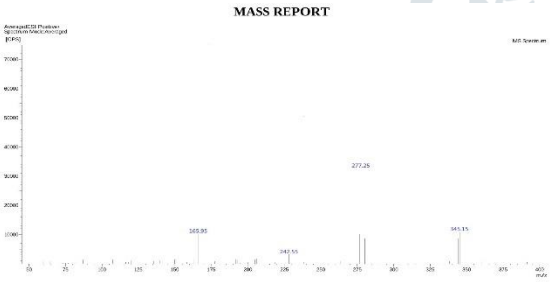


Fig. 4.3: Spectral data 5a2

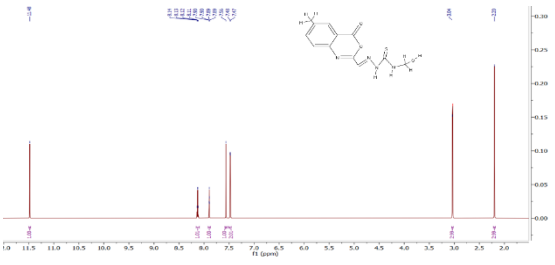


Fig. 4.4: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.a.2

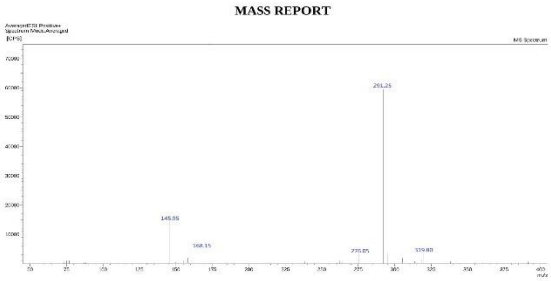


Fig. 4.5: Spectral data for 5a3

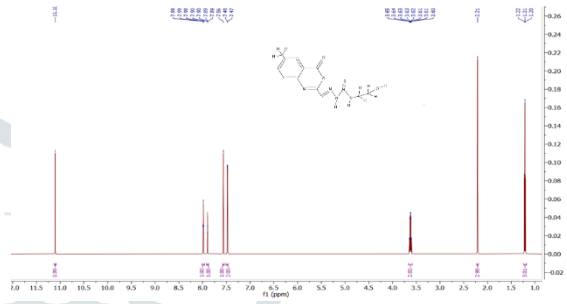


Fig. 4. 6: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.a.3

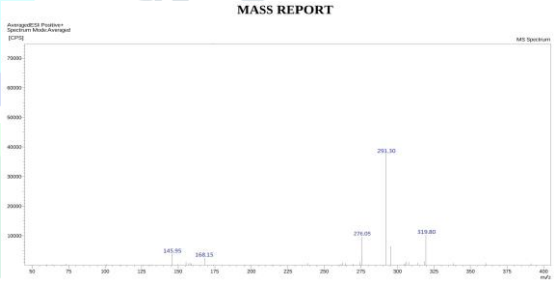


Fig. 4.7: Spectral data for 5.a.4

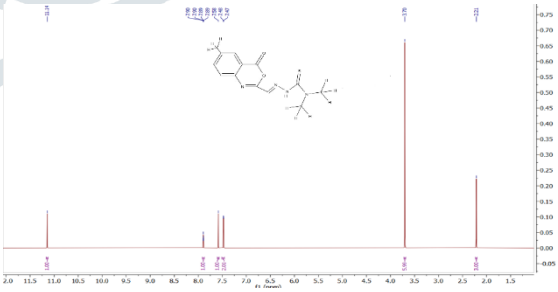


Fig.: 4.8: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.a.4

Spectral data for compound 5a5

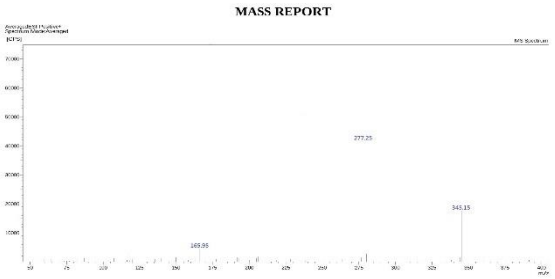


Fig. 4.9: Mass Spectral data 5.a.5

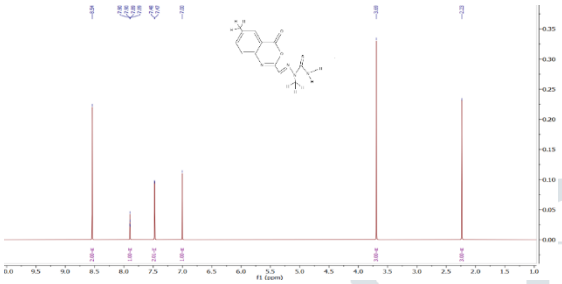


Fig: 4.10: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.a.5

Spectral data for compound 5b1

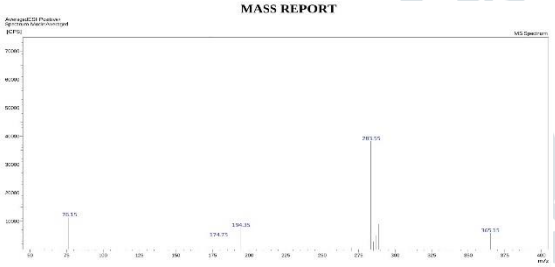


Fig. 4.11: Mass Spectral data 5.b.1

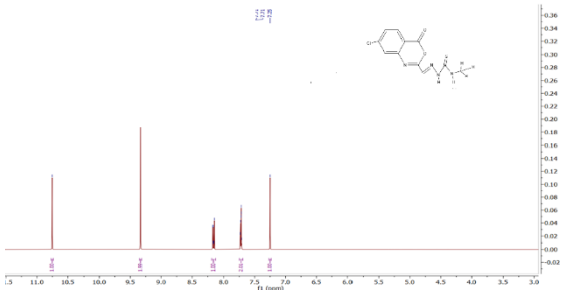


Fig: 4.12: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.b.1

Spectral data for compound 5b2

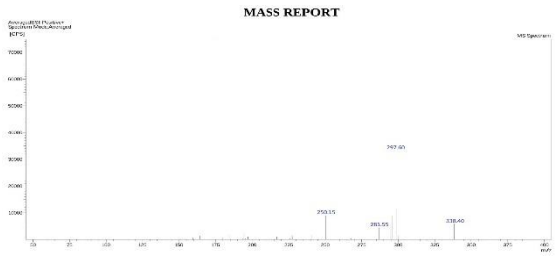


Figure 4.13: Mass Spectral data 5.b.2

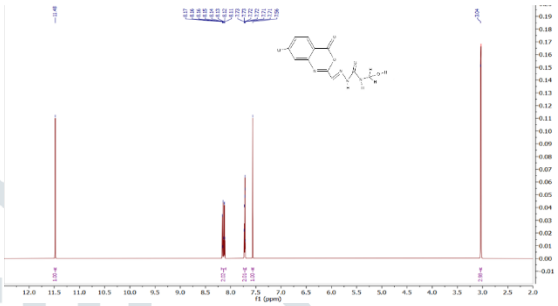


Fig: 4.14: ^{1}H N.M.R. Spectral data 5.b.2

Spectral data for compound 5b3

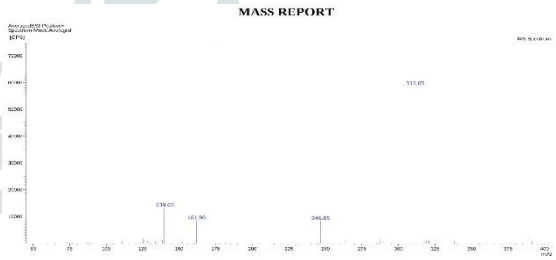


Fig. 4. 15: Spectral data 5.b.3

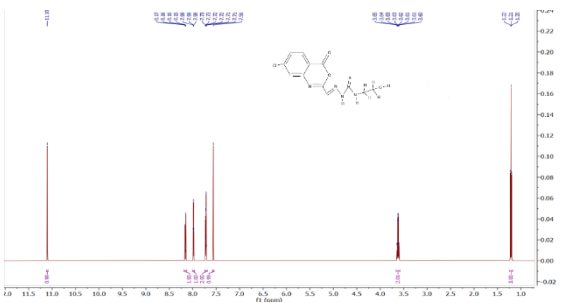
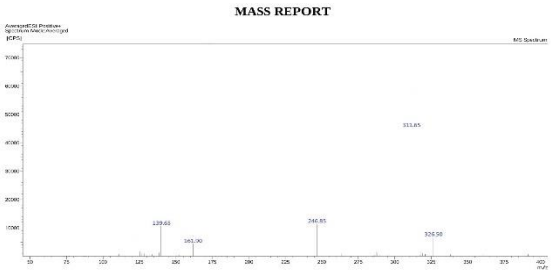


Fig: 4.16: ^{1}H N.M.R Spectral data .5.b.3

Spectral data for compound 5b4



Spectral, Data of compounds. 5c4

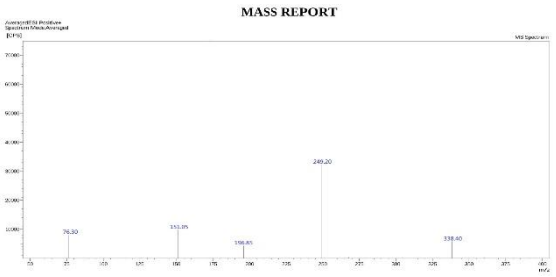


Fig. 4.21: Mass Spectral data 5.c.1

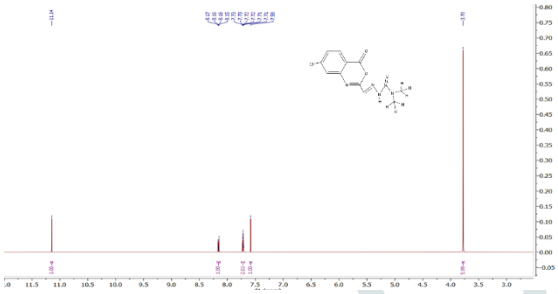


Fig. 4.18: ^{1}H N.M.R Spectral data 5.b.4

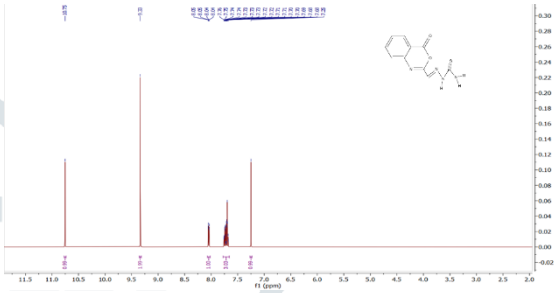


Figure 4.22: ^{1}H NMR Spectral data 5.c.1

Spectral data for compound 5b5

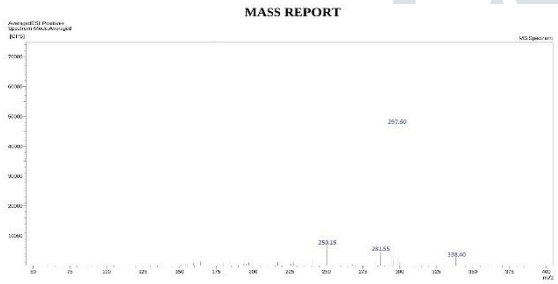


Fig. 4.19: Mass Spectral data 5.b.5

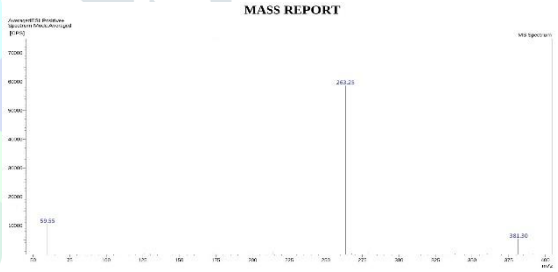


Fig. 4.23: Mass Spectral data 5.c.2

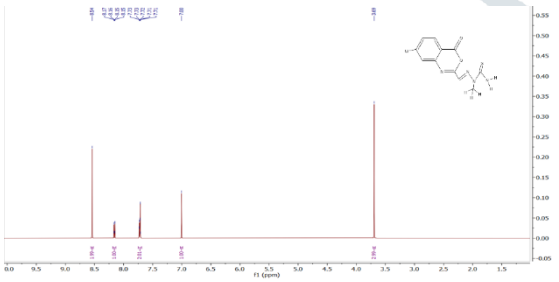


Fig. 4.20: H NMR Spectral data 5.b.5

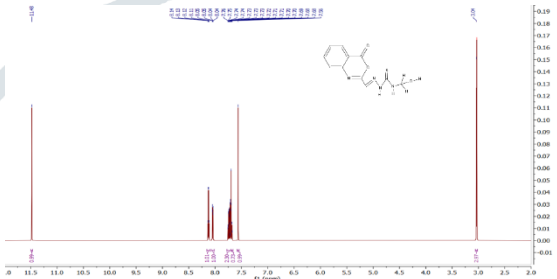


Fig: 4.24: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.c.2

Spectral data for compound 5c1

Spectral data for compound 5c3

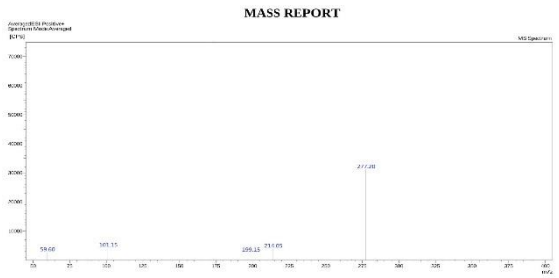


Fig: 4. 25. Mass Spectral data 5. c.3

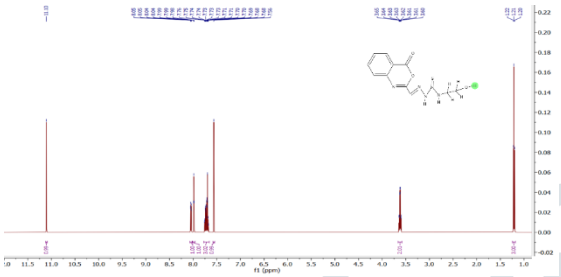


Fig: 4.26: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.c.3

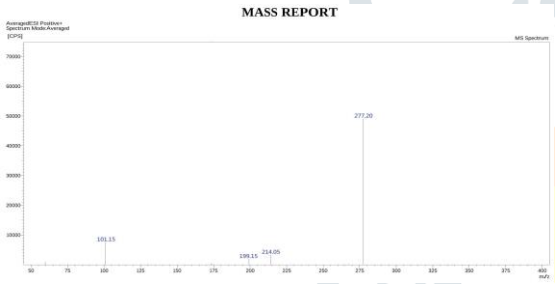


Fig: 4. 27. Mass, Spectral Data 5.c.4

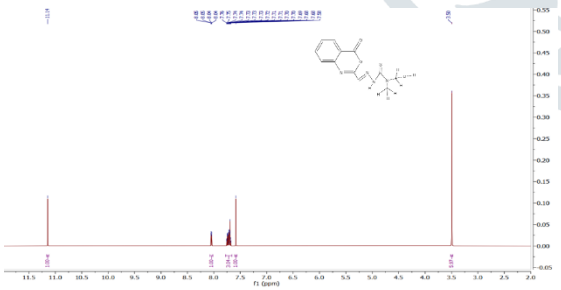


Fig: 4. 28: Proton Nuclear Magnetic Resonance (1H NMR) Spectral Data 5.c. 4

Spectral data for compound 5c5

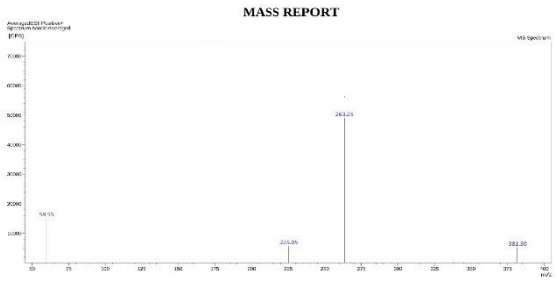


Fig. 4.29: Mass Spectral data 5.c.5

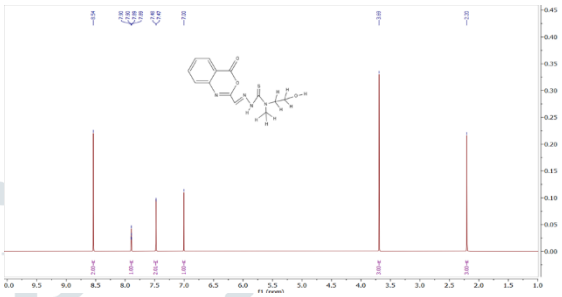


Fig. 4.30: ^{1}H NMR Spectral data 5.c.5

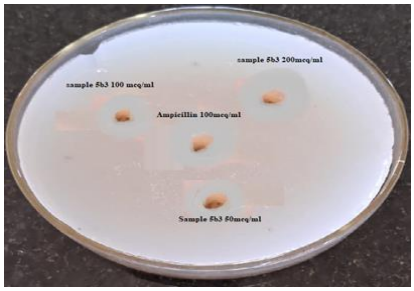
RESULTS OF ANTIMICROBIAL ACTIVITY

Invitro antibacterial activity

All the synthesized benzoxazinone-thiosemicarbazone derivatives were screened for antibacterial activity against both gram positive and gram negative bacterial strains. Antibacterial assay revealed the antibacterial potential of the all-tested compounds with difference in magnitude of inhibition of microbial growth. When compared to the reference antibiotic Ampicillin (100µg/ml) by disc-diffusion method, compounds **5b4, 5b4, 5c4** and **5c3** displayed good antibacterial potential against the selected gram positive and gram-negative strains. Compound **5b4** showed highest inhibition of bacterial growth against all the tested bacterial strains. From the results it is evident that chloro-substituted benzoxazinone-thiosemicarbazone derivatives possess better antibacterial activity than the methyl and un substituted derivatives. Results also implies that all the synthesized derivatives are more potent against the gram-positive bacteria than the gram-negative bacteria.

Table 2 Zone of inhibition (mm) of the compounds against gram positive bacteria

Compound	Gram positive bacteria					
	Bacillus subtilis			Staphylococcus aureus		
	50µg/ml	100µg/ml	200µg/ml	50µg/ml	100µg/ml	200µg/ml
5a$_1$	09	16	18	08	15	18
5a$_2$	10	17	19	12	16	20
5a$_3$	11	17	19	09	15	19
5a$_4$	11	21	23	13	16	21
5a$_5$	10	13	17	09	12	18
5b$_1$	12	17	19	10	15	21
5b$_2$	13	16	20	12	16	19
5b$_3$	17	25	30	16	23	26
5b$_4$	16	28	31	18	25	29
5b$_5$	13	21	23	08	13	17
5c$_1$	11	18	21	10	14	19
5c$_2$	13	17	21	11	16	19
5c$_3$	13	24	27	14	20	24
5c$_4$	15	25	29	15	22	26
5c$_5$	12	17	20	10	13	17
DMSO	3			2		
Ampicillin (100µg/ml)	32			30		



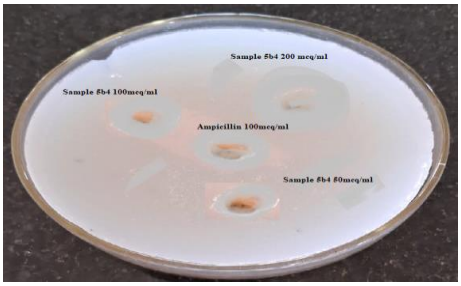
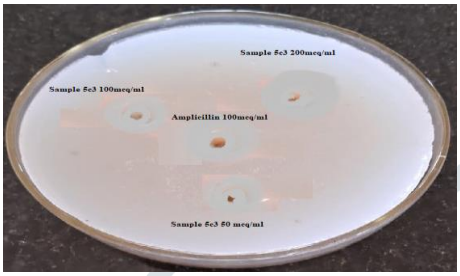


Fig. a. Sample 5b3 zone of inhibition on against gram Positive bacteria

Fig. a. Sample 5b4 zone of inhibition on against gram Positive bacteria



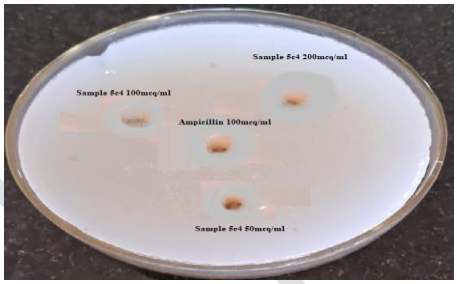
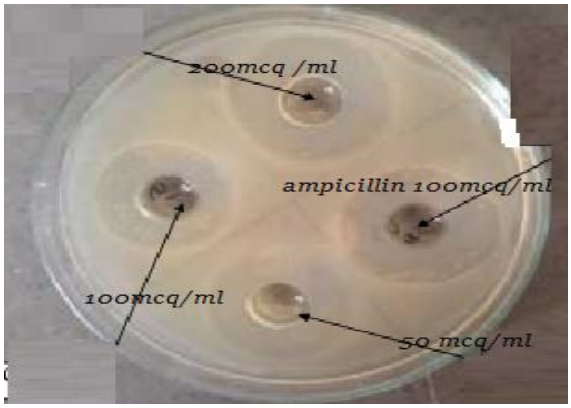


Fig. cSample 5c3 zone of inhibition on against gram Positive bacteria
Fig. d. Sample 5c4 zone of inhibition on against gram Positive bacteria

Table 3. Zone of inhibition (mm) of the compounds against gram negative bacteria

Compound	Gram negative bacteria					
	Escherichia coli			Pseudomonas aeruginosa		
	50µg/ml	100µg/ml	200µg/ml	50µg/ml	100µg/ml	200µg/ml
5a$_1$	09	13	15	08	13	14
5a$_2$	10	14	17	09	13	15
5a$_3$	11	14	17	10	14	15
5a$_4$	13	19	22	11	15	18
5a$_5$	06	12	14	09	12	14
5b$_1$	07	13	16	09	12	14
5b$_2$	09	14	17	10	15	18
5b$_3$	12	18	20	12	17	21
5b$_4$	14	18	23	14	19	23
5b$_5$	10	13	15	07	12	14
5c$_1$	11	15	18	09	13	16
5c$_2$	12	17	21	10	15	18
5c$_3$	14	21	25	13	20	24
5c$_4$	16	24	28	18	27	29
5c$_5$	07	12	15	09	13	16
DMSO	3			2		
Ampicillin (100µg/ml)	30			29		



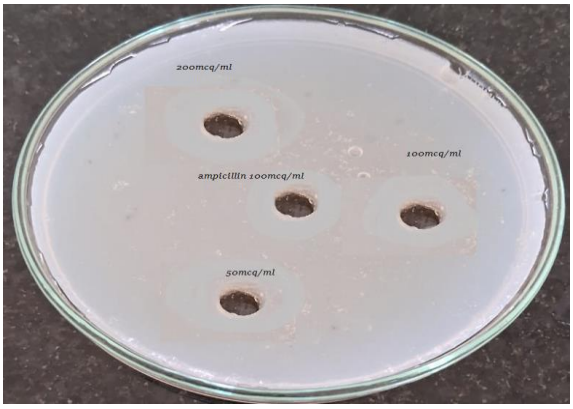
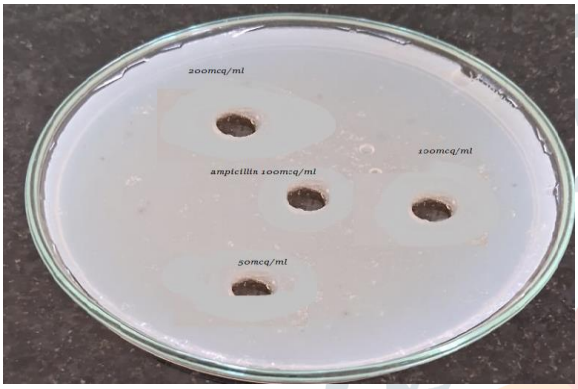


Fig. a. Sample 5b3 zone of inhibition on against gram negative bacteria
Fig. b. Sample 5b4 zone of inhibition on against gram negative bacteria



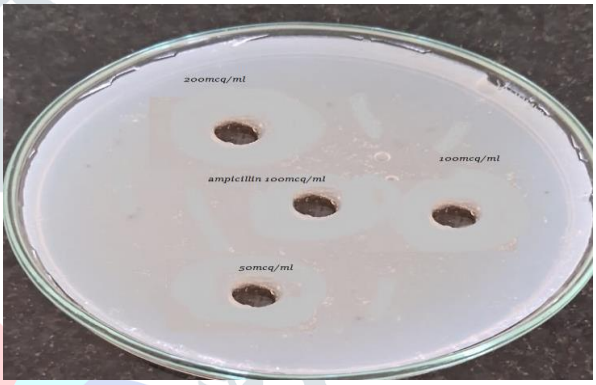


Fig. c. Sample 5c3 zone of inhibition on against gram negative bacteria
Fig. d. Sample 5c4 zone of inhibition on against gram negative bacteria

4.4. SUMMARY AND CONCLUSION

Versatile biological activity profile of the benzoxazinone scaffold attracted the medicinal chemists to explore lead molecules for the treatment of various microbial diseases. Extended literature avowed the indisputable antimicrobial properties of thiosemicarbazones that stimulated us to the current exploration.

Synthesis of novel benzoxazinone-thiosemicarbazone (**5a-5c**) hybrids from the condensation of substituted anthranilic acid (**1a-1c**), with acetyl chloride (**2**) to methyl bzoxazine-4-ones (**3a-3c**) that are oxidized using selenium dioxide to the corresponding aldehydes (**4a-4c**) followed by the condensation with various thiosemicarbazides.

Fifteen novel benzoxazinone-thiosemicarbazone **(5a1-5a5, 5b1-5b5 & 5c1-5c5)** were synthesized in adequate yields and characterization of the molecules was done by detailed spectral analysis using advanced analytical support. The titled compounds were screened for antibacterial and antifungal activities.

Results proclaimed that all the synthesized compounds were exhibiting antimicrobial properties. Compound**5b4** was contended to bear potent antimicrobial properties against the given bacterial. Further studies are needed to establish the possible mechanism of antibacterial and antifungal actions can helpful in the future development of benzoxazinone based thiosemicarbazone derivatives as novel antimicrobial agents.

REFERENCES

[1] Bain DI, Smalley RK (1968). Synthesis of 2-substituted-4H-3, 1-benzoxazin-4-ones, Journal of the Chemical Society C: Organic, 1593-1597.

[2] Bansal, Y.; Silakari, O. (2012) Bio. Med. Chem. 20, 6208.

[3] Besson. T, Rees. CB, Cottenceau G, (1996) Bioorg. Med. Chem. Lett., 6.

[4] Bhat. R, Pawar. P D (2008). Indian. Drugs. 45, 963.

[5] Brown, D. G. &Boström, J. (2016), Analysis of past and present synthetic methodologies on medicinal chemistry: where have all the new reactions gone J. Med. Chem. 59, 4443–4458.

[6] Chawla, A.; Kaur, R.; Goyal, A. J. Chem. (2011), 3, pp 457.

[7] Dahal R H, Shim,D S and Kim J (2017)"Development of actinobacterial resources for functional cosmetics," Journal of Cosmetic Dermatology, vol. 16, no. 2, pp. 243–252

[8] De Carvalho PGC, Ribeiro JM, Nakazato G, Garbin RPB, Ogatta SFY. Synthesis and Antimicrobial Activity of Thiohydantoins Derived from L-Amino Acids. Lett Drug Design Disc. 2020; 17(1): 94-102.

[9] Enumula, S., Pangal, A., Gazge, M., Shaikh, J.A., & Ahmed, K. (2014). Diverse Pharmacological aspects of Benzimidazole Derivatives. Research Journal of Chemical Sciences, 4(4), 78-88.

[10] Fair R.J, Tor Y (2014) "Antibiotics and bacterial resistance in the 21st century," Perspectives in Medicinal Chemistry, vol. 28, no. 6, pp. 25–64

[11] Galkina IV, Tudriy EV, Bakhtiyarova YV, Usupova LM, Shulaeva MP, Pozdeev OK, &Galkin VI, (2014). Synthesis and Antimicrobial Activity of Bis-4, 6-sulfonamidated 5, 7-Dinitrobenzofuroxans, Journal of Chemistry, 1–6.

[12] Galkina IV, Tudriy EV, Bakhtiyarova YV, Usupova LM, Shulaeva MP, Pozdeev OK, Galkin VI. (2014). Synthesis and Antimicrobial Activity of Bis-4, 6-sulfonamidated 5, 7-Dinitrobenzofuroxans. J Chem. 1–6.

[13] Gillespie S H, (2002), "Evolution of drug resistance in mycobacterium tuberculosis: clinical and molecular perspective," Antimicrobial Agents and Chemotherapy, vol. 46, no. 2, pp. 267–274, 2002.

[14] Huang, Q, Mao, J, Wan, B, Wang, Y, Brun, R, Franzblau, SG &Kozikowski, AP (2009), 'Searching for new cures for tuberculosis: Design, synthesis and biological evaluation of 2-methylbenzothiazoles', J. Med. Chem., vol. 52, no. 21, pp. 6757-6767.

[15] Huang, ST, Hsei, I, & Chen, C (2006), Synthesis and anticancer evaluation of bis (benzimidazoles), bis (benzoxazoles) and benzothiazoles', Bioorg. Med. Chem., vol. 14, no. 17, pp. 6106-6119.

[16] Jorgensen JH, Turnidge JD, Washington JA. (1999) Antibacterial susceptibility tests: dilution and disk diffusion methods. Mannual of Clinical Microbiology. 1526–1543, ASM Press, Washington, DC, USA, 7th edition,

[17] Maddila S, Gorle S, Seshadri N, Lavanya P and Jonnalagadda SB (2016), Synthesis, antibacterial and antifungal activity of novel benzothiazole pyrimidine derivatives. Arabian Journal of Chemistry, 9(5): 681-687.

[18] Mullard, A. (2017)the drug maker's guide to the galaxy. Nature 549, 445–447.

[19] N. Zanatta, D. M. Borchhardt, S. H. Alves, M. C. Squizani, T. M. Marchi, H. G. Bonacorso, M. P. Martins, Bio. Org. Med. Chem., 14, 3174 (2006).

[20] National Committee for Clinical Laboratory Standards (NCCLS), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Proposed standard NCCLS Document M27-p, NCCLS, Villanova, Pa, USA, 1992.

[21] Parameshwar Pabba, (2021) Antimicrobial Potential of Novel Benzoxazinone Derivatives tethered with Thiosemicarbazone Scaffold International journal of innovative research in technology, Vol.8 Issue 3.

[22] Parameshwar Pabba, (2022) Antimicrobial Potential of Novel Benzoxazinone derivatives tethered with Thiosemicarbazone scaffold, International journal of biology, Pharmacy and Allied sciences, November, 11(11)

[23] Parameshwar Pabba, (2022) Synthesis, characterization and antimicrobial evaluation of Novel Benzimidazole – Thiosemicarbazone hybrids, High Technology letters, Vol.28. Issue 3

[24] Parameshwar Pabba, (2023) Bioanalytical method process of Chromatographic analysis of tizanidine in the formulation and human plasma Rasayana journal of Chemistry Vol. 16 No. 1 422-427 January - March

[25] Ramesh. L S, Mahesh N, Jyoti WB, Wadekar. B, (2012). Internet. J. Pharm. Tec. Res., 4, 1653.

[26] Roughley, S. D. & Jordan, A. M. (2011), the medicinal chemist's toolbox: an analysis of reactions used in the pursuit of drug candidates. J. Med. Chem. 54, 3451–3479.

[27] S. D. Sayaji, B. P. Piste, (2013). Internet. J. chem. Tech. Res., 5, 2199.

[28] Saini, M S, Kumar A, Dwivedi J and Singh R,(2013), Int. J. Pharm. Sci. Res., 4(3), 66-77

[29] Sethi, R., Arora, S., Saini, D., & Jain, S. (2016). Synthesis, characterization, molecular docking studies and antimicrobial evaluation of N-benzimidazol-1-yl methyl benzamide derivatives. Indian Journal of Pharmaceutical Education and Research, 50(3), 424- 434.