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ANTIMICROBIAL POTENTIAL OF NOVEL BENZOXAZINONE DERIVATIVES TETHERED WITH THIOSEMICARBAZONE SCAFFOLD

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ABSTRACT

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 and fungal strains.

Keywords: Benzoxazin-4-one, Thiosemicarbazone, Antibacterial, Antifungal, Agar disc diffusion

INTRODUCTION

Antibiotic resistance in bacterial pathogens has currently reached very high and alarming levels. Indeed, according to a number of international bodies, this "antibiotic resistance crisis" could possibly bring us back to a "pre-antibiotic era" in the near future, if no effective actions are promptly undertaken **[1, 2]**. Unfortunately, the low success rate in antimicrobial agents' discovery and development observed during the last decades has led to a limited availability of new compounds for clinical use, thus exacerbating the burden of antibiotic resistance on morbidity and mortality rates. In this scenario, also many modern clinical practices associated with an increased risk of infection and in which antibiotics are essential (e.g., anti-cancer treatments, solid organ and stem cell transplantations, or implantation of prosthetic devices) are seriously at risk of success **[3]**.

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 **[4, 5]**. Benzoxazine moiety is present in several structures endowed with various activities, such as antiallergics **[6]**, antimicrobials **[7]**, anti-mycobacterials **[8]**, antifungals **[9]**. Besides their biological applications they can be used as starting materials for synthesis of 1,3,4-oxadiazoline-5-thione **[10]**, quinazolinone derivatives **[11]** and triazoles **[12]**.

Pervasive multidrug resistance of bacteria in the chemotherapy is the high priority challenge now a days to maintain public health **[13]**. According to the World Health Organization (WHO), by 2050, it is approximated that, the mortality may increase to 10 million every year. In virtue of unempirical practices in the antibiotic applications, the bacterial resistance has been escalated and increased the thrust to explore new antimicrobials synthetically **[14]**. Hence, the synthesis of Benoxazinone derivatives is of considerable interest due to their potent and significant biological activities and great pharmaceutical value. In the present study, we demonstrated the synthesis of a cluster of Benoxazinone based thiosemicarbazones and their antibacterial and antifungal activity on various gram positive, gram negative bacterial and fungal strains.

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.

Synthesis of Benzoxazinone thiosemicarbazone derivatives

General procedure for the synthesis of substituted benzoxazinones (3a-3c) [15]

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.

General procedure for the synthesis of substituted benzoxazinone carbaldehydes (4a-4c) [16]

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) [17]

New thiosemicarbazone hybrids (5a1-5, 5b1-5 and 5c1-5) were prepared by condensation of thiosemicarbazide, 4-methylthiosemicarbazide, 4-ethylthiosemicarbazide, 4- dimethyl

thiosemicarbazide, 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 solutions of different thiosemicarbazides (5.0 mmol) 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 recrystalized 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 unreacted material.

Antimicrobial Assay

Invitro 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)* andgram-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 **[18, 19]**.

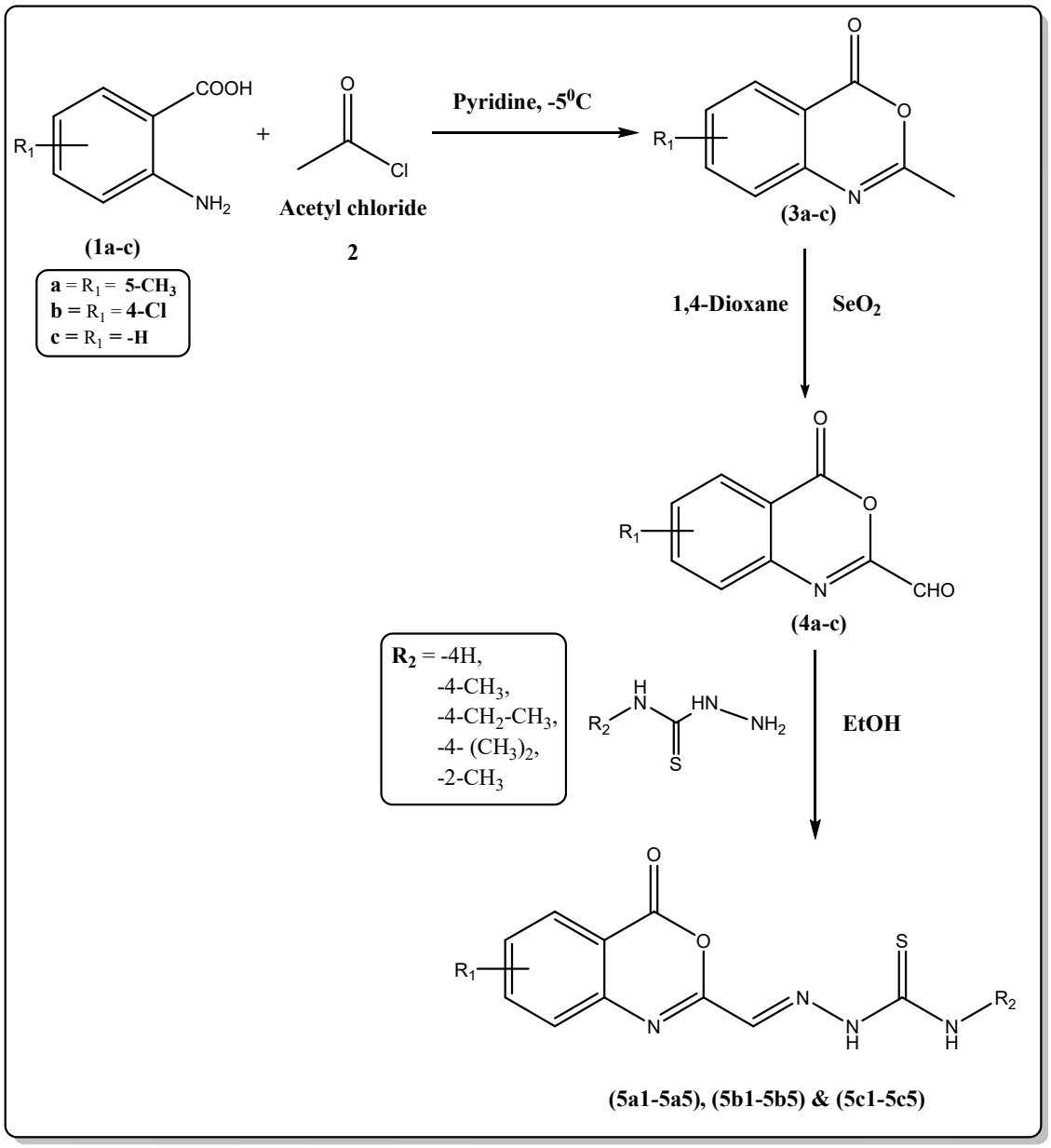


Figure 1: Scheme of synthesis of novel benzoxazinone-thiosemicarbazone hybrids

In vitro antifungal assay

The fungal strains were also 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 fungal strains (*Aspergillus niger, Candid albicans*). Nystatin (10µg/ml) in DMSO were used as reference antibiotics. Potato dextrose agar medium was taken in the pre-sterilized petri-dishes and the microorganisms were grown by inoculating the standard suspension of 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 potato dextrose agar medium and the petri-dishes were incubated at 28˚C for 48 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 **[20, 21]**.

RESULTS AND DISCUSSIONS

Chemistry

In this study, novel thiosemicarbazonederivatives containing the benzxoxazinone moiety were prepared according to reaction as in **Scheme 1 (Figure 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**).

Spectral data

(E)-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5a1)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 16012.5 (C=N), 3282.1 (N-H), 3056.2 (=C–H), 1292.4 (C-N),1541.6 (C=C), 1014.8 (C=S);**^{1}H NMR** (500 MHz, DMSO-d6) 1H NMR: δ 2.19 (3H, s), 7.48 (1H, dd, J = 8.2, 1.7 Hz), 7.60 (1H, dd, J = 8.2, 0.5 Hz), 7.66 (1H, dd, J = 1.7, 0.5 Hz), 7.75 (1H, s).**ESI-MS:** m/z Anal. Calcd. For $C_{11}H_{10}N_4O_2S$ ($[M + H]^+$): 262.29, found 263.25.

(E)-N-methyl-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5a2)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1608 (C=N), 3282.5 (N-H), 3059.4 (=C–H), 1290.5 (C-N),1544.1 (C=C), 1013.5 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.48 (s, 1H), 8.13 (q, J = 4.0 Hz, 1H), 7.92 – 7.87 (m, 1H), 7.56 (s, 1H), 7.48 (d, J = 1.2 Hz, 2H), 3.04 (s, 3H), 2.20 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{12}H_{12}N_4O_2S$ ($[M + H]^+$): 276.30, found 277.25.

(E)-N-ethyl-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5a3)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1606.2 (C=N), 3284.3 (N-H), 3063.5 (=C–H), 1294.5 (C-N),1542.3 (C=C),

1016.4 (C=S); **^{1}H NMR**(500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.99 (t, J = 3.0 Hz, 1H), 7.92 – 7.87 (m, 1H), 7.56 (s, 1H), 7.48 (d, J = 1.2 Hz, 2H), 3.62 (qd, J = 6.5, 2.9 Hz, 2H), 2.21 (s, 3H), 1.21 (t, J = 6.5 Hz, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{13}H_{14}N_4O_2S$ ([M + H]$^+$): 290.35, found 291.25.

(E)-N,N-dimethyl-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene) hydrazine-1-carbothioamide (5a4)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1606.5 (C=N), 3272.5 (N-H), 3068.4 (=C–H), 1292.5 (C-N),1544.6 (C=C), 1015.4 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.14 (s, 1H), 7.92 – 7.87 (m, 1H), 7.58 (s, 1H), 7.48 (d, J = 1.2 Hz, 2H), 3.70 (s, 6H), 2.21 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{13}H_{14}N_4O_2S$ ([M + H]$^+$): 290.35, found 291.30.

(E)-1-methyl-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5a5)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1608.8 (C=N), 3276.2 (N-H), 3057.2 (=C–H), 12885 (C-N),1538.2 (C=C), 1013.8 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.54 (s, 2H), 7.89 (q, J = 0.9 Hz, 1H), 7.48 (d, J = 1.2 Hz, 2H), 7.00 (s, 1H), 3.69 (s, 3H), 2.23 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{12}H_{12}N_4O_2S$ ([M + H]$^+$): 276.30, found 277.25.

(E)-2-((7-chloro-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5b1)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1594.5 (C=N), 3275.4 (N-H), 3068.3 (=C–H), 1285.7 (C-N),1540.5 (C=C), 1015.5 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 10.75 (s, 1H), 9.33 (s, 2H), 8.18 – 8.13 (m, 1H), 7.75 – 7.69 (m, 2H), 7.25 (s, 1H).**ESI-MS:** m/z Anal. Calcd. For $C_{10}H_7ClN_4O_2S$ ([M + H]$^+$): 282.70, found 283.55.

(E)-2-((7-chloro-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)-N-methylhydrazine-1-carbothioamide (5b2)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1601.5 (C=N), 3273.5 (NH), 3063.2 (=C–H), 1287.2 (C-N),1544.8 (C=C), 1018.5 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.48 (s, 1H), 8.18 – 8.10 (m, 2H), 7.75 – 7.69 (m, 2H), 7.56 (s, 1H), 3.04 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{11}H_9ClN_4O_2S$ ([M + H]$^+$): 296.70, found 297.60.

(E)-2-((7-chloro-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)-N-ethylhydrazine-1-carbothioamide (5b3)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1607.3 (C=N), 3264.8 (N-H), 3055.3 (=C–H), 1294.5 (C-N),1538.4 (C=C), 1015.8 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.10 (s, 1H), 8.18 – 8.13 (m, 1H), 7.99 (t, J = 3.0 Hz, 1H), 7.75 – 7.69

(m, 2H), 7.56 (s, 1H), 3.62 (qd, J = 6.5, 2.9 Hz, 2H), 1.21 (t, J = 6.5 Hz, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{12}H_{11}ClN_4O_2S$ ([M + H]$^+$): 310.76, found 311.65.

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

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1610.4 (C=N), 3275.3 (N-H), 3060.4 (=C–H), 1293.8 (C-N),1538.2 (C=C), 1016.4 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.14 (s, 1H), 8.18 – 8.13 (m, 1H), 7.75 – 7.69 (m, 2H), 7.58 (s, 1H), 3.78 (s, 6H).**ESI-MS:** m/z Anal. Calcd. For $C_{12}H_{11}ClN_4O_2S$ ([M + H]$^+$): 310.76, found 311.65.

(E)-2-((7-chloro-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)-1-methylhydrazine-1-carbothioamide (5b5)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1609.2 (C=N), 3283.5 (N-H), 3061.2 (=C–H), 1298.5 (C-N),1544.2 (C=C), 1019.3 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.54 (s, 2H), 8.18 – 8.13 (m, 1H), 7.75 – 7.69 (m, 2H), 7.00 (s, 1H), 3.69 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{11}H_9ClN_4O_2S$ ([M + H]$^+$): 296.70, found 297.60.

(E)-2-((4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5c1)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1603.8 (C=N), 3284.6 (N-H), 3065.3 (=C–H), 1287.2 (C-N),1542.3 (C=C), 1016.2 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 10.75 (s, 1H), 9.33 (s, 2H), 8.05 (dd, J = 6.9, 1.7 Hz, 1H), 7.78 – 7.66 (m, 3H), 7.25 (s, 1H). **ESI-MS:** m/z Anal. Calcd. For $C_{10}H_8N_4O_2S$ ([M + H]$^+$): 248.26, found 249.20.

(E)-N-methyl-2-((4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5c2)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1600.8 (C=N), 3278.4 (N-H), 3060.3 (=C–H), 1292.4 (C-N),1539.5 (C=C), 1014.7 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.48 (s, 1H), 8.13 (q, J = 4.0 Hz, 1H), 8.05 (dd, J = 6.9, 1.7 Hz, 1H), 7.78 – 7.70 (m, 2H), 7.70 – 7.66 (m, 1H), 7.56 (s, 1H), 3.04 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{11}H_{10}N_4O_2S$ ([M + H]$^+$): 262.29, found 263.25.

(E)-N-ethyl-2-((4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5c3)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1604.5 (C=N), 3276.3 (N-H), 3055.8 (=C–H), 1291.9 (C-N),1540.2 (C=C), 1017.5t (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.10 (s, 1H), 8.05 (dd, J = 6.9, 1.7 Hz, 1H), 7.99 (t, J = 3.0 Hz, 1H), 7.78 – 7.66 (m, 3H), 7.56 (s, 1H), 3.62 (qd, J = 6.5, 2.9 Hz, 2H), 1.21 (t, J = 6.5 Hz, 3H).**ESI-MS:** m/z Anal. Calcd. For

$C_{12}H_{12}N_4O_2S$ ([M + H]$^+$): 276.31, found 277.20.

(E)-N,N-dimethyl-2-((4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5c4)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1605.1 (C=N), 3285.5 (N-H), 30602.6 (=C–H), 1292.1 (C-N),1545.2 (C=C), 1019.5 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 11.14 (s, 1H), 8.05 (dd, J = 7.0, 1.8 Hz, 1H), 7.78 – 7.66 (m, 3H), 7.58 (s, 1H), 3.50 (s, 6H).**ESI-MS:** m/z Anal. Calcd. For $C_{12}H_{12}N_4O_2S$ ([M + H]$^+$): 276.31, found 277.20.

(E)-1-methyl-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5c5)

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1606.3 (C=N), 3286.8 (N-H), 3065.2 (=C–H), 1289.3 (C-N),1539.2 (C=C), 1014.2 (C=S); **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.54 (s, 2H), 7.92 – 7.87 (m, 1H), 7.48 (d, J = 1.2 Hz, 2H), 7.00 (s, 1H), 3.69 (s, 3H), 2.20 (s, 3H).**ESI-MS:** m/z Anal. Calcd. For $C_{12}H_{12}N_4O_2S$ ([M + H]$^+$): 276.31, found 277.25.

All the synthesized compounds were resulted in competitive yields as reported in **Table 1**.

Antimicrobial activity

Invitro antibacterial activity

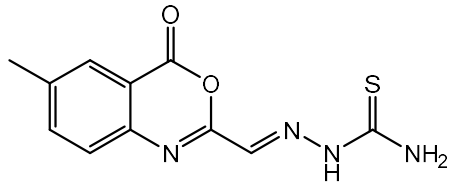
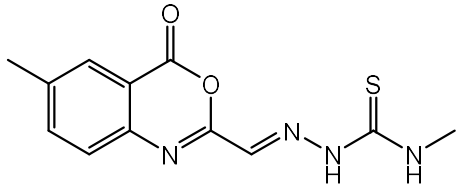
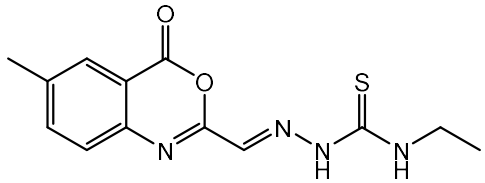
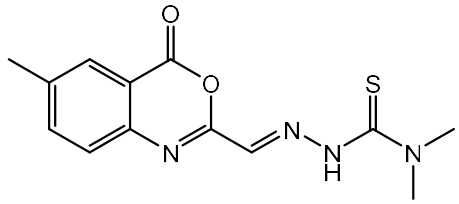
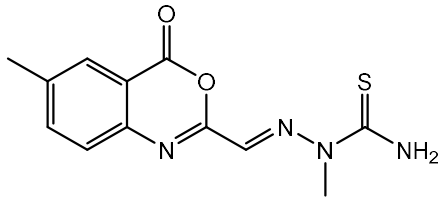
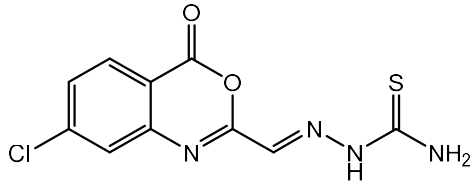
All the synthesized benzoxazinone-thiosemicarbazone derivatives were screened for antibacterial activity against both gram positive (**Table 2**) and gram negative (**Table 3**) bacterial strains. Antibacterial assay revealed the antibacterial potential of the all-tested compounds with difference in 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 methyland unsubstituted derivatives. Results also implies that all the synthesized derivatives are more potent against the gram-positive bacteria than the gram-negative bacteria.

Invitro antifungal activity

Antifungal profile for the prepared compounds was also developed against selectedfungal strains (**Table 4**). Interestingly, **5a** series compounds showed significant antifungal activity than **5b**and **5c**series against all fungal strains. Compounds **5a3** and **5a2** displayed

noticeable antifungal activity relative to the reference standard nystatin. It is also observed that *Aspergillus niger*is more sensitive to the prepared compounds than other strains. **5a3** is the most potentantifungal among all the derivatives and showed maximum growth inhibition with 23 mm of zone of inhibition against *Aspergillus niger* followed by **$5b_4$ and$5b_1$**. All compounds displayed less sensitivity towards *Candida albicans than Aspergillus niger*.

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

Comp. No	R_1	R_2	Structure	Mol. Form.	m.p in °C	% Yield
$5a_1$	**$-CH_3$**	**-H**		**$C_{11}H_{10}N_4O_2S$**	**213-214**	**74**
$5a_2$	**$-CH_3$**	**$-CH_3$**		**$C_{12}H_{12}N_4O_2S$**	**221-222**	**78**
$5a_3$	**$-CH_3$**	**$-C_2H_5$**		**$C_{13}H_{14}N_4O_2S$**	**229-230**	**82**
$5a_4$	**$-CH_3$**	**$-(CH_3)_2$**		**$C_{13}H_{14}N_4O_2S$**	**219-220**	**79**
$5a_5$	**$-CH_3$**	**$-CH_3$**		**$C_{12}H_{12}N_4O_2S$**	**211-212**	**80**
$5b_1$	**-Cl**	**-H**		**$C_{10}H_7ClN_4O_2S$**	**227-228**	**78**

$5b_2$	**-Cl**	**$-CH_3$**		**$C_{11}H_9ClN_4O_2S$**	**223-224**	**81**
$5b_3$	**-Cl**	**$-C_2H_5$**		**$C_{12}H_{11}ClN_4O_2S$**	**241-242**	**77**
$5b_4$	**-Cl**	**$-(CH_3)_2$**		**$C_{12}H_{11}ClN_4O_2S$**	**234-235**	**74**
$5b_5$	**-Cl**	**$-CH_3$**		**$C_{11}H_9ClN_4O_2S$**	**236-237**	**69**
$5c_1$	**H**	**-H**		**$C_{10}H_8N_4O_2S$**	**254-255**	**81**
$5c_2$	**H**	**$-CH_3$**		**$C_{11}H_{10}N_4O_2S$**	**246-247**	**79**
$5c_3$	**H**	**$-C_2H_5$**		**$C_{12}H_{12}N_4O_2S$**	**238-239**	**80**

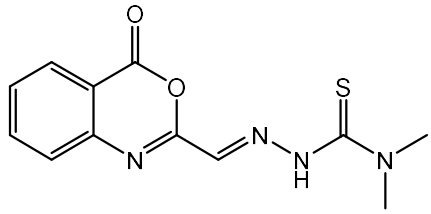
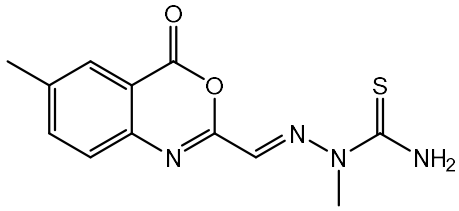
$5c_4$	**H**	**$-(CH_3)_2$**		**$C_{12}H_{12}N_4O_2S$**	**231-232**	**74**
$5c_5$	**H**	**$-CH_3$**		**$C_{10}H_{10}N_4S_2$**	**259-260**	**74**

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**		

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**		

Table 4: Zone of inhibition (mm) of the compounds against fungal strains

Compound	*Fungal strains*					
	Aspergillus niger			*Candida albicans*		
	50µg/ml	100µg/ml	200µg/ml	50µg/ml	100µg/ml	200µg/ml
$5a_1$	11	14	18	10	15	18
$5a_2$	13	16	21	13	17	20
$5a_3$	13	19	23	14	19	21
$5a_4$	10	12	15	10	13	16
$5a_5$	07	10	12	09	13	15
$5b_1$	09	12	14	08	10	13
$5b_2$	09	11	14	09	11	13
$5b_3$	07	10	12	08	10	11
$5b_4$	08	10	11	07	11	12
$5b_5$	06	08	09	05	07	10
$5c_1$	10	13	15	08	11	13
$5c_2$	08	11	12	09	11	14
$5c_3$	09	11	13	10	12	13
$5c_4$	07	12	14	10	13	14
$5c_5$	08	11	13	09	11	14
DMSO	2			3		
Nystatin (10µg/ml)	21			25		

CONCLUSION

In conclusion, synthesis of a novel series of benoxazinone-thiosemicarbazone hybrids **(5a1-5a5, 5b1-5b5 & 5c1- 5c5)** were synthesized using commercially available starting materials and economically feasible methods. The structure elucidation was done with the help of their physical, analytical, and spectral data. All the 15 compounds were screened for *Invitro* antimicrobial activity was carried out using both gram positive, gram negative bacterial and fungal strains (**Table 2-4**) using disc diffusion methods. The average zone of inhibition was measured and compared with the standard drugs, showing significant antimicrobial activities. Compounds 5b4 and 5c4 displayed highest activity against the tested gram-negative and gram-positive strains. Compounds 5a3 displayed noticeable activity against the tested fungal strains. 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.

Conflict of Interest

Authors disclose no conflict of interest

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