Antimicrobial Potential of Novel Benzoxazinone Derivatives Tethered with Thiosemicarbazone Scaffold

[1*]Priyadarshini Mandru, [2 DR.] Pabba Parameshwar
[1]*Research Scholar Career Point University, Kota, Rajasthan.*
[2]*Research Supervisor, Dept. of Chemistry, Career Point University, Kota, Rajasthan*
[*]*Corresponding author: Priyadarshini Mandru*

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.

Key words: 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 the field 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)* 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 [18, 19].

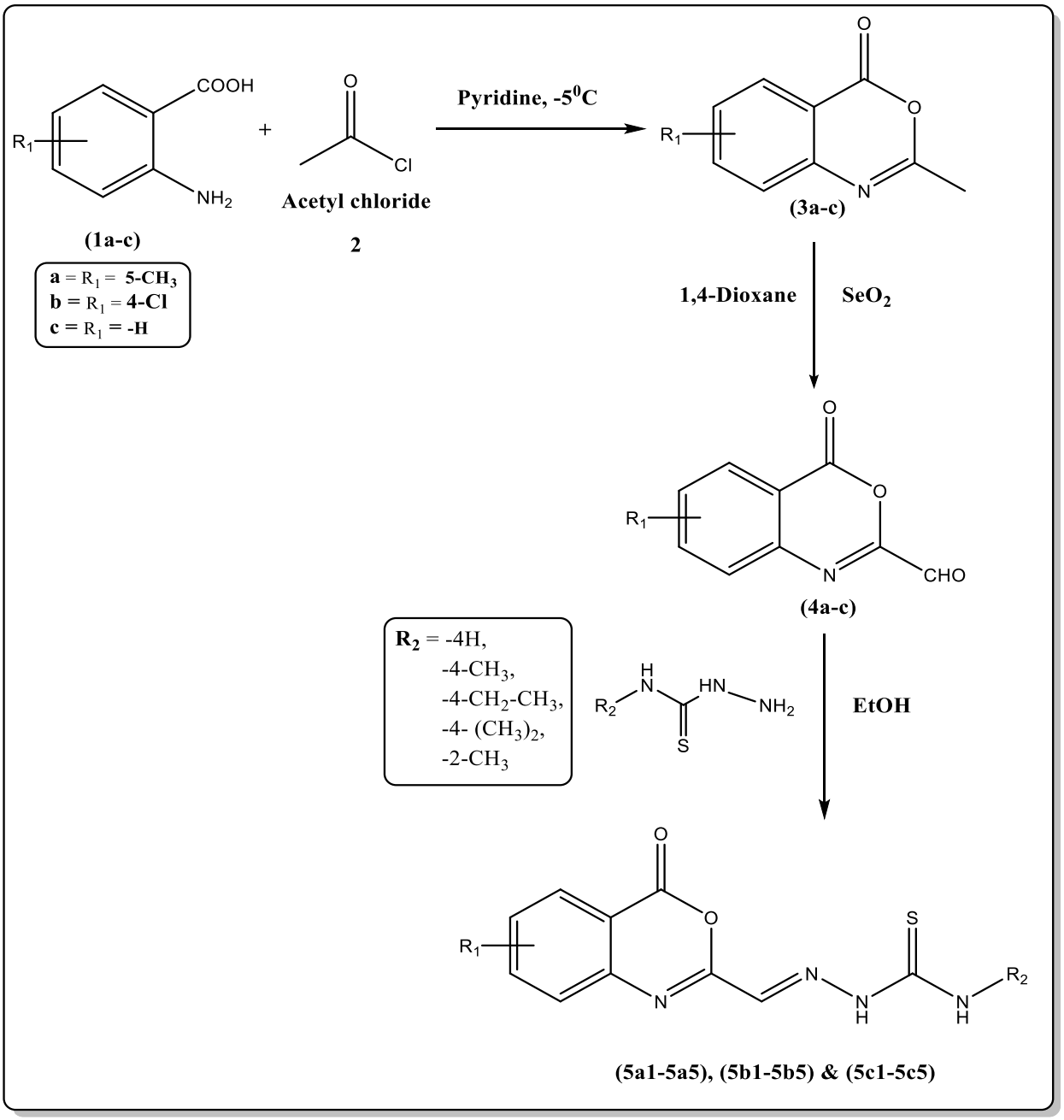


Figure 1**.** Scheme of synthesis of novel benzoxazinone-thiosemicarbazone hybrids

Invitro 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 thiosemicarbazone derivatives 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)

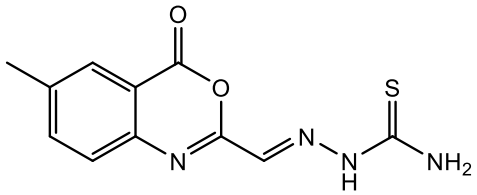
Pale yellow crystals, IR (KBr): ν_{max} in cm^{-1}: 1605.1 (C=N), 3285.5 (N-H), 30602.6 (=C–H), 1292.1 (C-N),1545.2 (C=C), 1019.5 (C=S); ^{1}H NMR (500 MHz, DMSO-d6) δ 11.14 (s, 1H), 8.05 (dd, J = 7.0, 1.8 Hz, 1H), 7.78 – 7.66 (m, 3H), 7.58 (s, 1H), 3.50 (s, 6H). ESI-MS: m/z Anal. Calcd. For $C_{12}H_{12}N_4O_2S$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

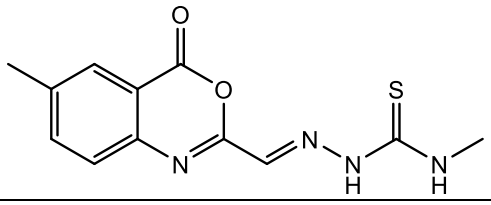
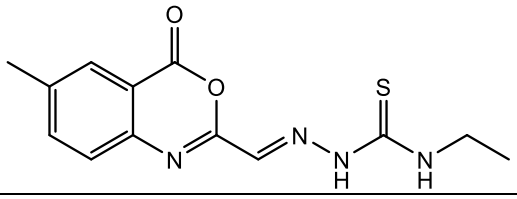
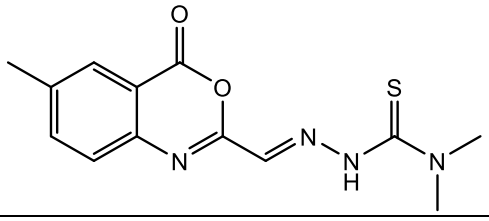
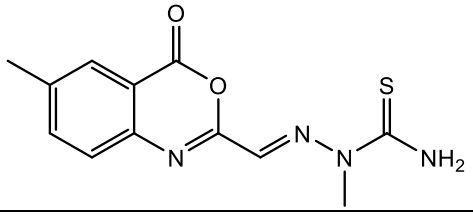
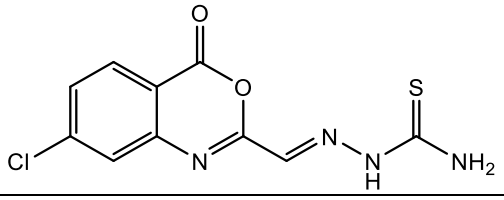
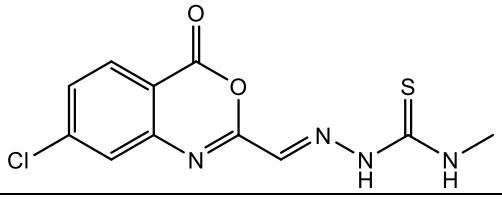
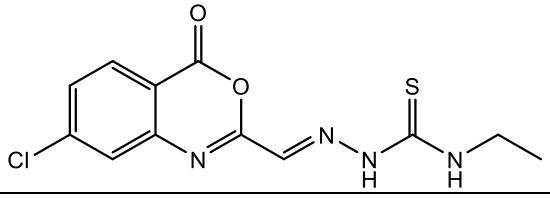
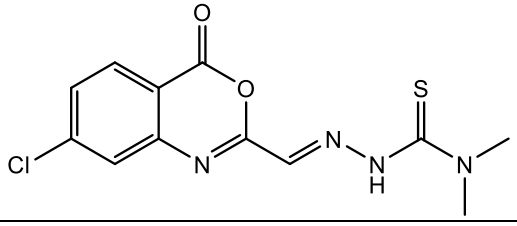
(E)-1-methyl-2-((6-methyl-4-oxo-4H-benzo[d][1,3]oxazin-2-yl)methylene)hydrazine-1-carbothioamide (5c5)

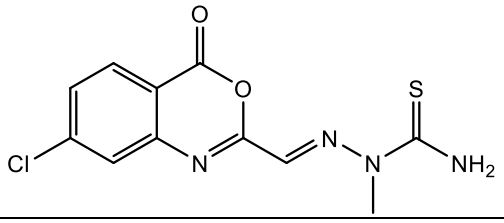
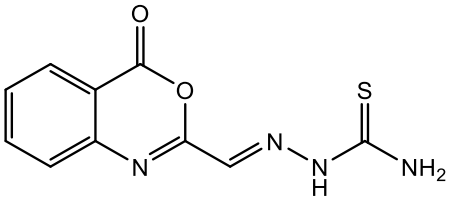
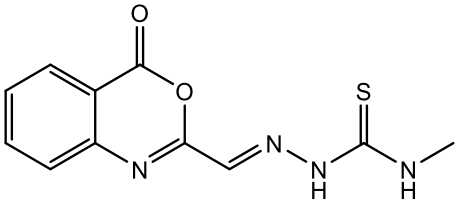
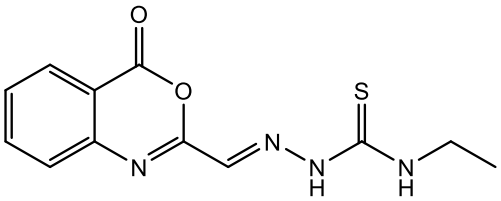
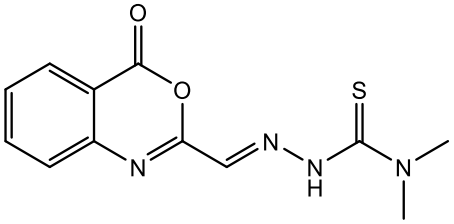
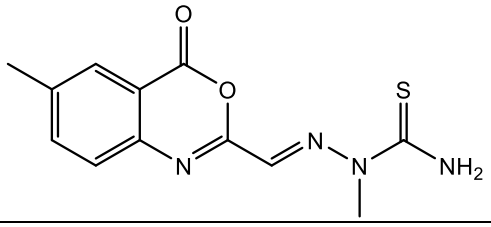
Pale yellow crystals, IR (KBr): ν_{max} in cm^{-1}: 1606.3 (C=N), 3286.8 (N-H), 3065.2 (=C–H), 1289.3 (C-N),1539.2 (C=C), 1014.2 (C=S); ^{1}H NMR (500 MHz, DMSO-d6) δ 8.54 (s, 2H), 7.92 – 7.87 (m, 1H), 7.48 (d, J = 1.2 Hz, 2H), 7.00 (s, 1H), 3.69 (s, 3H), 2.20 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{12}H_{12}N_4O_2S$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

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

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

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 methyl and unsubstituted 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		

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		

Invitro antifungal activity

Antifungal profile for the prepared compounds was also developed against selected fungal 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 potent antifungal 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 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

REFERENCES

[1] O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. [(accessed on 22 November 2019)]; 2016 May; Available online: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.

[2] Interagency Coordination Group on Antimicrobial Resistance No Time to Wait: Securing the Future from Drug-Resistant Infections Report to the Secretary-General of the United Nations. [(accessed on 22 November 2019)];2019 Apr; Available online: https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_final_report_EN.pdf?ua=1.

[3] Hutchings M, Truman A, Wilkinson B, Antibiotics: Past, present and future. Current Opinion in Microbiology, 51, 2019, 72–80.

[4] Khan ZA, Naqvi SAR, Shahzad SA, Mahmood N, Yar M, Zahoor AF, Asian Journal of Chemistry, 25, 2013, 152–156.

[5] Shreder K, Hu Y, Fraser A, Kohno Y, Kojima A, Ishiyama J, Akihiko K, Junichi I, Yasu K, Ishiyama, Y. US7879846-B2, 2011.
[6] Loev B, Jones H, Brown RE, [1,4]benzoxazin-2,3-dione as antiallergic agents. Journal of Medicinal Chemistry, 28, 1985, 24–27.
[7] Babenysheva A, Lisovskaya N, Belevich I, Lisovenko N, Synthesis and antimicrobial activity of substituted benzoxazines and quinoxalines, Pharmaceutical Chemistry Journal, 40, 2006, 611–613.
[8] Li X, Liu N, Zhang H, Knudson SE, Slayden RA, Tonge PJ, Synthesis and SAR studies of 1,4-benzoxazine MenB inhibitors: novel antibacterial agents against Mycobacterium tuberculosis, Bioorganic and Medicinal Chemistry Letters, 20, 2010, 6306–6309.
[9] Fringuelli R, Giacche N, Milanese L. Bulky 1,4-benzoxazine derivatives with antifungal activity, Bioorganic and Medicinal Chemistry Letters, 17, 2009, 3838–3846.
[10] M.F. Ismail, S.A. Emara and O.E.A. Mustafa, Phosphorus, Sulfur, Silicon Rel. Elem., 63, 373 (1991).
[11] Madkour HMF, Reactivity of 4H-3,1-benzoxazin-4-ones towards nitrogen and carbon nucleophilic reagents: applications to the synthesis of new Heterocycles, Arkivoc, 36, 2004, 36-54.
[12] Deshmukh MB, Suryawanshi AW, Mali AR, Desai SRD, Synthetic Communications, 34, 2004, 2655-2662.
[13] Sardari S, Feizi S, Rezayan AH, Azerang P, Shahcheragh SM, Ghavami G, Habibi A. Synthesis and Biological Evaluation of Thiosemicarbazide Derivatives Endowed with High Activity toward *Mycobacterium Bovis*. Iranian journal of pharmaceutical research, 16(3), 2017, 1128-1140.
[14] Kobayashi RKT and Nakazato G (2020) Editorial: Nanotechnology for Antimicrobials. Front. Microbiol. 11:1421
[15] Bain DI, Smalley RK, Synthesis of 2-substituted-4H-3,1-benzoxazin-4-ones, Journal of the Chemical Society C: Organic, 1968, 1593-1597.
[16] Han X, Peng B, Xiao BB, Sheng-Li Cao, Yang CR, Wang WZ, Wang FC, Li HY, Yuan XL, Shi R, Liao J, Wang H, Li J, Xu X. Synthesis and evaluation of chalcone analogues containing a 4-oxoquinazolin-2-yl group as potential anti-tumor agents. European journal of medicinal chemistry, 162, 2019, 586-601.
[17] Mouayed A. Hussein, Muhammad Adnan Iqbal, Muhammad Ihtisham Umar, Rosenani A. Haque, Teoh Siang Guan. Synthesis, structural elucidation and cytotoxicity of new thiosemicarbazone derivatives, Arabian Journal of Chemistry, 12(8), 2019, 3183-3192.
[18] Galkina IV, Tudriy EV, Bakhtiyarova YV, Usupova LM, Shulaeva MP, Pozdeev OK, Galkin VI, Synthesis and Antimicrobial Activity of Bis-4,6-sulfonamidated 5,7-Dinitrobenzofuroxans, Journal of Chemistry, 2014, 1–6.
[19] De Carvalho PGC, Ribeiro JM, Nakazato G, Garbin RPB, Ogatta SFY. Synthesis and Antimicrobial Activity of Thiohydantoins Derived from L-Amino Acids. Letters in Drug Design and Discovery, 17(1), 2020, 94-102.
[20] Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. Mannual of Clinical Microbiology. 1526–1543, ASM Press, Washington, DC, USA, 7th edition, 1999.
[21] 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.