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Synthesis of New Benzoxazole Derivatives and Evaluation of their Antifungal and Antibacterial Activities

Vaishali Bhardwaj [a++*] and Anurag Sharma [b++]

[a] Department of Pharmacy, Rajshree Group of Institutions, Pilibhit Road Bareilly, UP, 243122, India.
[b] Invertis Institute of Pharmacy, Invertis University Bareilly, UP, 243123, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Analytical chemistry aims to synthesize some novel benzoxazole derivatives with lower side effects and more efficacy. In this research, we synthesized the target compound's reaction of 2-aminophenol in methanol and added carbon disulfide to obtain benzo[d] oxazole-2-thiol. This obtained compound again reacts with 4-chlorobenzoic acid to form 4-(benzo [d]oxazol-2-ylthio) benzoic acid. 4-(benzo[d]oxazol-2-ylthio) benzoic acid was further treated with substituted esters to give the crude product (4a-4e). The structures of derivatives are characterized with the help of IR, 1H NMR, TLC Spectroscopy, and melting point. The derivatives of the compounds were tested for antibacterial & antifungal activities. Antimicrobial evaluation was performed individually with gram-positive and gram-negative bacteria S. aureus and Pseudomonas aeruginosa. Aspergillus niger and Candida albicans were the two types of fungi on which antifungal activities were conducted. The outcomes were compared to those of the common medications Amphotericin B and

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[++] Assistant Professor;
**Corresponding author: Email: anuragsrms1996@gmail.com, anuragrockbly@gmail.com;*

Ciprofloxacin. It was discovered that the produced compounds have considerable antibacterial and antifungal activity. Four synthesized derivatives are named as 4 (d, b, e, c). The 4 d showed a good antimicrobial activity against the S. aureus. The 4 c showed good antifungal activities against the Candida albicans MTCC3541. The order of antifungal activity of these synthesized compounds is 4c >4e > 4b >4d. The standard. The 4 c showed a good antifungal activity against the C. albicans.

Keywords: Benzoxazoles; antimicrobial; antifungal; alcohols; ciprofloxacin; amphotericin B; methanol.

1. INTRODUCTION

A variety of organic compounds can be synthesized with the help of heterocyclic compounds. These substances are widely used in the creation of new medications; in the pharmaceutical sector, heterocyclic compounds account for almost 80% of the finest medications. The majority of naturally occurring active agrochemicals and medicines are heterocyclic [1,2]. A heterocyclic chemical, benzoxazole (1-Oxa-3-aza-1H-indene) has a benzene fused oxazole ring structure; an oxazole is a 1-3-azole with an oxygen atom and a nitrogen atom of the pyridine type at the 3-position in the five-membered ring [3]. In the pharmaceutical industry, lead compounds with proven activity are typically molecularly modified to find new medications regularly. Molecular alteration has the potential to increase the activity that combines distinct groups with equivalent action in a single compound by changing, adding, or removing a moiety from the original lead molecule. According to a review of the literature, one effective way to create novel medications is by molecular alteration in drug design [4]. Oxazole derivatives are referred to as "isosteres of natural nucleotide" and have been the subject of numerous studies aimed at creating synthetic analogs with significant chemotherapeutic effects [5]. Numerous naturally occurring and artificially synthesized bioactive compounds with benzoxazole scaffold have been shown to exhibit a broad range of biological activities, such as anti-viral, anti-microbial, anti-leishmanial, anti-malarial, and inhibition of the activity of eukaryotic topoisomerase II enzyme as well as activity against cancer cells that are resistant to multiple drugs [6]. As a result, the need to create novel compounds as possible therapeutic agents is more important than ever. A significant class of chemicals under investigation are benzoxazole derivatives. The benzoxazole skeleton has been linked to numerous biological activities in recent years, making it one of the most significant scaffolds present in pharmacologically active substances [7]. The biological characteristics of benzoxazole heterocyclic compounds have been extensively explored due to their immense interest [8]. Benzoxazole nucleus modifications have produced a wide range of molecules with different pharmacological properties. Because of the potential uses they may have in the medical area, researchers have long been interested in the synthesis, structure, and biological activity of benzoxazole derivatives. The benzoxazole moiety is becoming more and more important in the pharmaceutical field. It has been extensively researched to investigate its pharmacological support in various pharmacological situations. These novel benzoxazole generations' biological profiles show significant advancements over those of earlier compounds. Given the significance of the benzoxazole moiety in medicine, it makes sense to create some novel benzoxazole derivatives and test them for biological activity. There have been some intriguing advancements in the biological activity of benzoxazole derivatives in recent years noteworthy advancements in the biological activities of derivatives of benzoxazole have been observed [9]. Derivatives of benzoxazole exhibit a wide range of pharmacological actions. Consequently, benzoxazole has taken up a special position in the field of medicinal chemistry. There are a few instances of the benzoxazole ring system in nature. In scientific studies, benzoxazole is used as a starting material to synthesize bigger, typically bioactive substances [10]. It shares structural similarities with isosteres of naturally occurring cyclic nucleotides like adenine and guanine which is why it probably interacts with biopolymers in the living systems and shows diverse biological activities like antimicrobial, anti-inflammatory, and analgesic, antifungal, anticonvulsants, antitumor, anticancer, CNS activities, antihyperglycemic activity, anti-tubercular, anti-HIV agents, anthelmintic and other anticipated activities [11]. The use of antibiotics has increased in the past years around the world. The global antibiotic use increase by 65% was found between the years 2000-2015. An increase in the consumption of antibiotics also impacts the overall health of the world. By 2030, the total amount of antibiotics used worldwide will reach

128 billion if countries continue to utilize them at their current annual growth rates. Additionally, it is well-recognized that broad-spectrum antibiotics account for the majority of antibiotic usage [12]. Treatment failures, mortality, and costs are rising due to the inappropriate and extensive use of antibiotics [13]. The need for novel antimicrobial agents becomes evident when one considers the widespread usage of antibiotics along with the rise in drug resistance to them [14,15].

2. MATERIALS AND METHODS

For the synthesis of new benzoxazole derivatives, Marck and Himedia provided all of the analytical grade chemicals and reagents. Newly distilled solvents were used for the synthesis and purification. The malting points of synthetic compounds were found using a melting point instrument owned by Royal Scientific. The reaction's completion was monitored using silica gel G thin plates. Using JNM-ECS 400 MHz, the ^{1}H NMR and ^{13}C NMR spectra were captured. Chemical changes in δ (ppm) were detected in NMR using the internal standard TMS. Compound spectra were recorded using a Shimadzu FTIR spectrometer.

2.1 General Procedure for the Synthesis of Synthesis of Benzo[d]oxazole-2-thiol

Benzo[d]oxazole-2-thiol was obtained by mixing 1.1 g of 2-aminophenol with 15 ml of methanol, adding 0.7 g of potassium hydroxide in 3 ml of water, and then adding 0.9 ml of carbon disulfide. The mixture was then refluxed at 65 °C for 5 hours. After the reaction was finished, the mixture was poured into water, which was neutralized with concentrated hydrochloric acid. The solid separation was filtered and washed with hexane, recrystallized with ethanol, and dried to yield the pure compound.

2.2 Synthesis of 4-(benzo[d]oxazol-2-ylthio) Benzoic Acid

A solution of 1.56 gm of chlorobenzoic acid and 1.51 gm of benzoxazole-2-thiol (II) in dry THF (30 ml) was agitated with 2 ml of triethylamine for 4-6 hours at room temperature. TLC was used to track the reaction (chloroform: methanol/9:1, Rf 0.82). Following the reaction's conclusion, THF was eliminated, and 30 milliliters of ice-cold water were stirred into the residue. To obtain a crude product, the solid precipitate was filtered, cleaned with hexane and water, recrystallized with ethanol, and dried [16].

2.3 Synthesis of Target Compounds (4a-4e)

“4-(benzo[d]oxazol-2-ylthio) benzoic acid” (2.5 gm) dissolved in 20 ml ethanol in a clean and dried 250 ml round bottom flask .0.10 mole of substituted esters were added. refluxed the reaction for 6 hours. The reaction residue was filtered to obtain the crude product (4a-4e).

3. CHEMISTRY

The synthesis of Benzoxazole derivatives was prepared by condensation of carboxylic acid and then reacting the resulting product with Benzo[d]oxazole-2-thiol and finally derivatives were prepared by refluxing substituted 4 – chloro benzoic acid with 4-(benzo[d]oxazol-2-ylthio) benzoic acid. In this study, as anti-microbial drugs, some new Benzoxazole derivatives were produced.

3.1 Methyl 4- (benzo[d]oxazol-2-ylthio) Benzoate (4a)

IR (KBr-Cm-1); 3375 (str, N-H), 3000 (str, C-H), 2400 (str, C-S), 1644 (str, C= C Ar), 1508(str, O-N), 1281 (str, C - N), 1095 (str, C - O), 1019 (str, C=O). ^{1}H NMR (DMSO); δ 8.0-6.8 (d, 6H, Ar-H), 4.6 (s, 5H, COOH), 3.8 (s, ^{1}H, NH)

3.2 Ethyl 4- (benzo[d]oxazol-2-ylthio) Benzoate 4 (b)

IR (KBr Cm-1); 3375 (str, N-H), 3001 (str, C-H), 2400 (str, C-S), 1750 (str, C=O), 1508 (str, O-N) 1267 (str, C-N Ar), 1085 (str, C-O) .^{1} H NMR (DMSO); δ 7.8-7.2 (d, 8H, Ar - H), 5.4 (s, 2 H, COOH), 4.6 (s, 3H, NH)

3.3 Butyl 4- (benzo[d]oxazol-2-ylthio) Benzoate 4 (c)

IR (KBr- Cm -1); 3362 (str, N-H), 3010 (str, C-H), 2400 (str, C-S), 1636(str, C-C), 1750 (str,C=O), 1700 (str, C=N),1507 (str, O-N) 1300 (str, C-O). ^{1} H NMR (DMSO) δ; 8.0-6.40 (8H, Ar-H), 6.40 (d, 5H, COOH), 4.0 (s, 4H, NH)

3.4 Propyl 4- (benzo[d]oxazol-2-ylthio) Benzoate 4 (d)

IR (KBr Cm^{-1}); 3320 (str, N-H), 3000 (str, C-H), 2410 (str, C-S), 2410 (str, C-S),1750 (str, C=O),

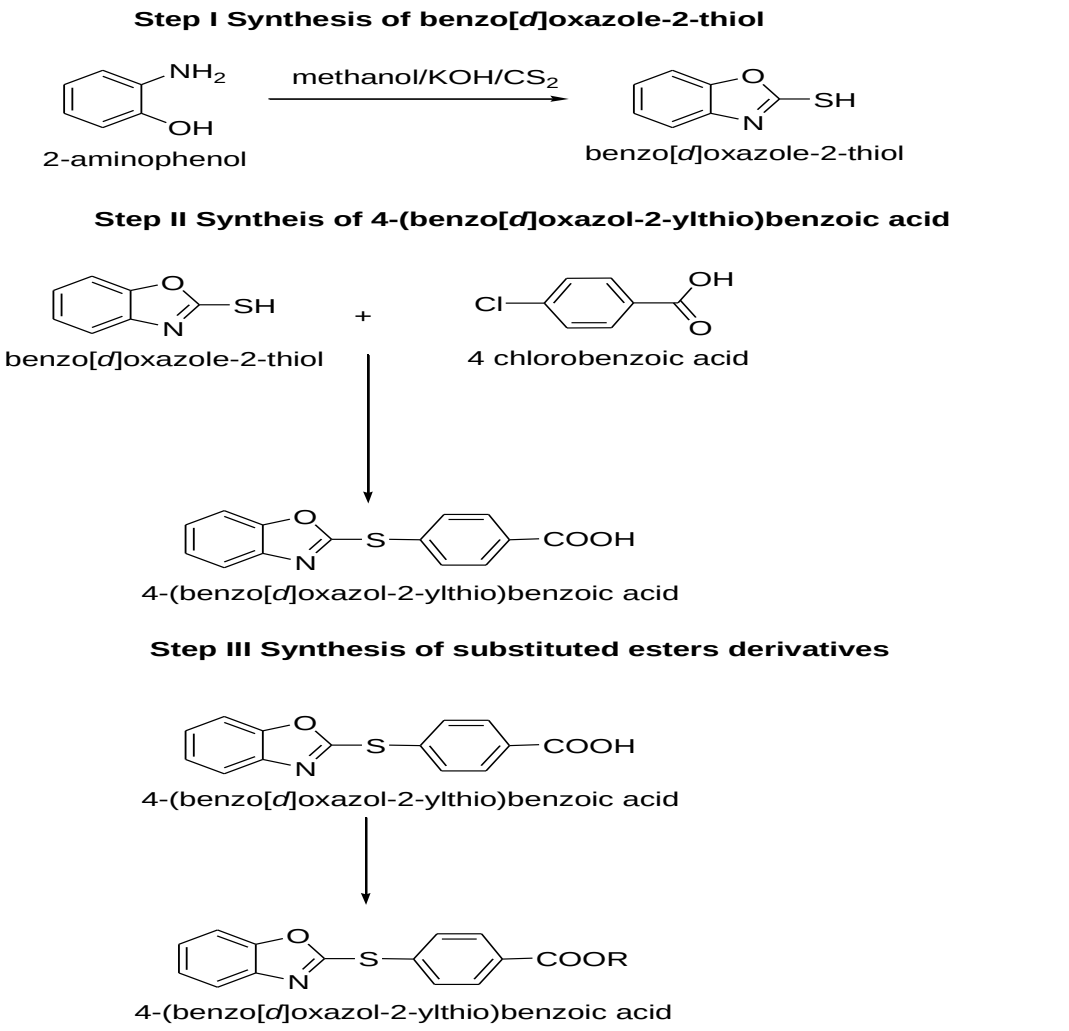


Fig 1. Chemical structure

Table 1. Reaction conditions of different benzoxazole derivatives

S. No.	Product	R	Time (h)	R_f Value	Yield (%)
1	4a	Methyl	5	0.80	75
2	4b	Ethyl	6	0.70	80
3	4c	Butyl	4	0.61	68
4	4d	n- propanol	5.5	0.87	85
5	4e	Isopropyl alcohol	6	0.74	90

1508 (str, O-N), 1210 (str, C-O), 1430 (str, C-C). ^{1}H NMR (DMSO); 7.8-6.5 (d, 8H, Ar-H), 5.0 (m, 4H, COOH), 4.1 (s, 3H, NH)

3.5 Isopropyl 4-(benzo[d]oxazol-2-ylthio) benzoate 4 (e)

IR (KBr-Cm^{-1}); 3330 (str, N-H), 3010 (str, C-H), 2430(str , C-S), 1507 (str, O-N), 1304 (str, C-N),1114 (str, C-O) 1^{H} NMR (DMSO); δ 7.9-7.0 (d, 8H, Ar-H), 5.0 (m, 3H, COOH), 4.0(s, ^{1}H , NH)

4. BIOLOGICAL EVALUATION

4.1 Antibacterial Activity

By using the Zone Inhibition Method, the antibacterial activity was examined (Kirby-Bauer method). A bacterial culture of S. aureus

(adjusted to 0.5 McFarland Unit - Approx cell density - 1.5 X 108 CFU/mL) was spread out on 100 µl of Mueller-Hinton agar (MHA) plates. Next, discs containing 10 µl of various concentrations (0 to 100 mg/ml) were placed. To obtain the necessary quantity to be placed onto the disc, 10% of the sample was extracted and serially diluted. One disk per plate was loaded with solvent alone, acting as a vehicle control, and one disc containing 10µg of ciprofloxacin was used as a positive control. The S. aureus plates were incubated for 24 hours at 37°C (Basil Scientific Corp. India). A space cleared out to surround the disc was measured and recorded [17].

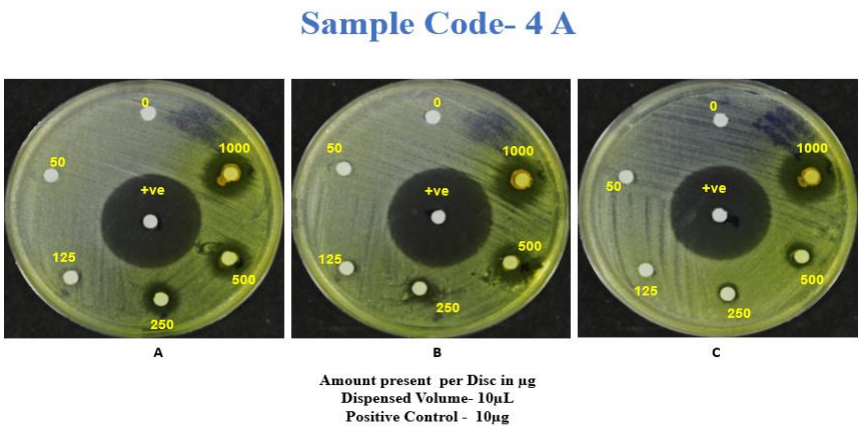
4.2 Antifungal Activity

By using the Zone Inhibition Method, the antifungal activity was examined (Kirby-Bauer method). After spreading 100 µl of Candida albicans fungal culture (adjusted to 0.5 McFarland Unit - Approx cell density, 1.5 X 108 CFU/mL), the discs containing 10 µl of various concentrations (0 to 100 mg/ml) were placed on the SDA (Sabouraud Dextrose Agar) plates. Each plate had one disc filled solely with solvent, acting as the vehicle control, and one disc containing 50µg of amphotericin B was used as the positive control. The Candida albicans plates were incubated for 24 hours at 37 °C in an incubator provided by Basil Scientific Corp. India. Measurements and records were made of the cleared areas that surrounded the disc [18].

5. RESULTS AND DISCUSSION

Two gram-negative bacteria were used to test each synthesized compound's antibacterial properties. (S.aureus MTCC96 and Pseudomonas aeruginosa MTCC3541) using ciprofloxacin as a control. The minimal inhibitory concentrations (MICs) are the lowest concentrations that inhibit the growth of the bacteria. Four synthesized derivatives are named as 4 (d, b, e, c). The 4 d showed a good antimicrobial activity against the S. aureus MTCC96 the order of antimicrobial activity of the synthesized compounds is 4c>4e>4b>4d. The standard deviations of the following compounds are 0.577, 0.601, 0.571, and 0.582 respectively. Some synthesized compounds were evaluated their antimicrobial activity concerning Pseudomonas aeruginous MTCC3541. 4 a showed good antimicrobial activity against S. aureus the order antimicrobial activity of these synthesized compounds is 4b>4d>4e>4a. The standard deviations of the following compounds are 0.571, 0.623, 0.578, and 0.588 respectively**.**

The antifungal activity of each of the newly discovered compounds was also examined against two different fungus species: Aspergillus niger MTCC281 and Candida albicans MTCC3541. As a control, amphotericin B was employed. The lowest inhibitory concentration (MIC) represents the antifungal action**.** Four synthesized derivatives are named as 4 (d, b, e, c). The 4-d showed good antifungal activities against the Candida albicans MTCC3541. The order of antifungal activity of these synthesized compounds is 4c >4e > 4b >4d. The standard deviations of the following compounds are 0.577, 0.601, 0.571, and 0.582 respectively. The same synthesized compounds were evaluated its antifungal activities concerning Aspergillus niger MTCC281. 4 b showed good antifungal activities against the Aspergillus niger the order of antimicrobial activity of these synthesized compounds is 4a>4d>4e>4b. The standard deviations of the following compounds are 0.573, 0.623, 0.576, and 0.583 respectively.



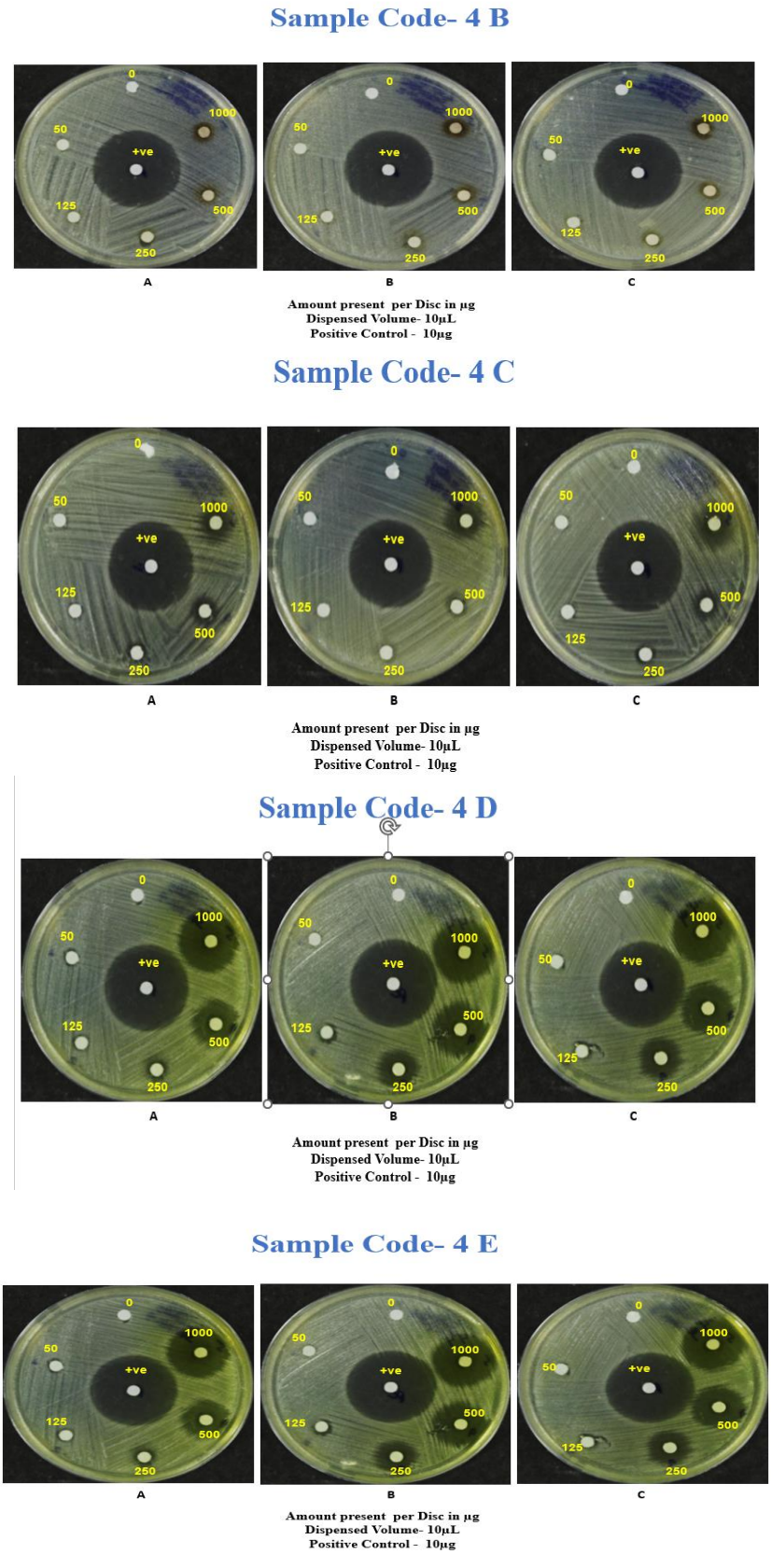


Fig. 2. Zone diffusion test *S. aureus*

6. CONCLUSION

Considering the scope of new synthesized benzoxazole derivatives in drug discovery and their importance in the medicinal field. The present work is focused on synthesis characterization and their biological evaluation as antimicrobial & antifungal studies of target molecules to our knowledge. Some new routes for the synthesis of Benzoxazole derivative have been devised and the target molecule has been screened for selected biological activities. The characterization of the synthesized compounds as Infrared spectroscopy, melting point, thin layer chromatography, and ^{1} H NMR. The target molecules, benzo[d]oxazole-2-thiol, were created by reacting 2-aminophenol with methanol and then adding carbon disulfide. After a second reaction with 4-chlorobenzoic acid, the obtained product yields 4-(benzo[d]oxazol-2- ylthio) benzoic acid. Substituted esters were used to further treat 4-(benzo[d]oxazol-2-ylthio) benzoic acid, yielding the target compounds (4a-4e).

Percentage yield obtained for compounds (4a-4e) was 65-90%. These novel synthesized structures were determined using IR and NMR spectroscopy. Thin layer chromatography and the compounds' sharp melting points verified the homogeneity and purity of each mixture. The production of the synthesized compounds and, consequently, the accuracy of the predicted structures drawn for the synthesized compounds were positively validated by all of the aforementioned outcomes. The antibacterial and antifungal properties of the target compounds were investigated. One gram-positive bacteria, S. aureus, and one gram-negative bacteria were the targets of the antimicrobial evaluation. Aeruginosa pseudomonas. In these investigations, ciprofloxacin was the usual medication. Two fungi, such as Aspergillus niger and Candida albicans, were used to evaluate the antifungal properties. Amphotericin B is a common medication. The findings of these investigations indicate that several recently synthesized compounds have strong antifungal and antibacterial properties against gram-positive bacteria and fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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