Design, synthesis, and biological evaluation of N-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5 yl) sulfonyl) piperazin-1-yl) acetamide as antimicrobial agents.

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Abstract:

Microbial contamination is increasing day by day due to the resistance to the existing drugs to mitigate this need a new series of N-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5 yl) sulfonyl) piperazin-1-yl) acetamide are designed, synthesized, and evaluated for antimicrobial activity. Benzofuran, Piperazine, and Sulfonyl contain many pharmacologically important compounds, which is presently marketed. The synthesized derivatives were characterized by IR, NMR, and MS spectroscopy. The novel compounds were screened for in vitro anti-bacterial and anti-fungal activities. The selected compounds showed comparable activity with respect to the standard. The compounds A, B, and D exhibited good antibacterial and antifungal activities compared to the reference drugs Amoxicillin and Fluconazole against the selected strains. Other compounds also shown the activity against microbes but some are more active on either bacteria or fungi. To get a potent compounds it must be active on all strains of microbial activity.

Keywords: Antibacterial activity, antifungal activity, benzofuran, and Sulfonyl-Piperazine.

1. Introduction

Nearly a century has passed since the invention of the microbe contact. The primary aggressors that governed the host-pathogen relationship and produced disease were once thought to be microbes. Later, increased knowledge of what characteristics of bacteria and their hosts have led to the realization in the host-pathogen relationship does not necessarily result in disease. [1] Microbial infection is the result of the entrance of infectious agents into the organisms, the growth of these agents, and the host tissue's response to these pathogens. [2]. Microbial contamination was determined to be responsible for 7.7 million deaths worldwide in 2019. This equates to one-eighth of all global deaths. [3]. Microorganisms can become resistant to antibiotics through any of the following processes: selection, mutation, phage transduction, and transference, but environmental exposure can also lead to the development of microbial resistance. [4]. Antimicrobial resistance is developing due to the growth and spread of drug-resistant bacteria with emerging resisting techniques, endangering the effectiveness of currently available medications to treat common diseases. One of the biggest challenges in the study of antibacterial and fungicides is the development of fresh chemical substances to combat resistant fungus and bacteria. This is motivating researchers to develop novel, powerful antibacterial and antifungal medicinal medicines. [5].For over 40 years, the macrolide class of antimicrobial agents has been widely used to treat various infectious conditions. They have been particularly useful as a treatment for patients who are allergic to penicillin and are effective against pneumococcal, streptococcal, and mycoplasma infections [6]. The naturally occurring cationic antimicrobial peptides (AMPs) present in almost all higher forms of life are one promising class of antibiotics [7]. Proteolytic instability nevertheless, and occasionally tiresome synthetic processes. Due to the emergence, dissemination, and persistence of multi-drug-resistant bacteria, antimicrobial resistance (AMR) is a global public health concern that threatens the health of people, animals, and the environment [8]. Nowadays, it is believed that the effects of antimicrobial resistance on people are comparable to those of climate change [9]. Heterocyclic compounds are significant compounds in medicinal chemistry as their promising pharmacological action. Heterocyclic rings containing oxygen, nitrogen, and sulfur become a member of a five- or six-membered heterocyclic group is highly significant. because of their major effect on clinical application in medicinal chemistry[10]. The heterocyclic compounds, benzofuran is an important class of compounds that occupied its place in numerous bioactive natural products benzofuran-anchored drugs play an important role in the treatment of various types of diseases, and new benzofuran derivatives with medicinal value are being actively

explored worldwide. It is well known by the generic term benzofuran and is conveniently eliminated in order to define it. Benzofuran derivatives exhibit significant activity against several viruses comprising analgesic, antiparasitic, anticancer, anti-HIV, anti-protozoal, antitubercular, anti-inflammatory, anticonvulsant, and anti-HIV [11]. The basic structure of various biologically active substances, including antioxidant, anti-inflammatory, analgesic, anti-plasmodial, and anti-amoebic medicines, is composed of condensed benzofuran with a pyrimidine scaffold. Additionally, they act as 5-lipoxygenase and angiotensin II converting enzyme inhibitors. [12[Previous studies found that benzofuran-imidazole analogue derivatives show good cytotoxic effects on the MCF-7 cell line [13].

Along with the benzofuran six-membered heterocyclic ring piperazine has a wide range as an antimicrobial agent. Piperazine is a heterocyclic atom and which is belongs to the family of medicines called anthelmintic. Anthelmintics are used in the treatment of worm infections. Piperazines derivatives have also been shown to be a large number of antibiotics that contain amide linkage. Several derivatives of amides were prepared and found to possess antimicrobial activities. A literature survey reveals that various drugs e.g. penicillin (antibacterial), pyrazinamide (anti-tubercular), indinavir, ritonavir, etc.(protease inhibitors as anti-AIDS) contain their particular activities due to the amide linkage present in their structures [14]. The definitions of virulence and pathogenicity have altered throughout time as microbiologists have tried to express that microbial pathogenesis represents an interaction between two entities, host and pathogen, in a recent examination of these concepts. [15].

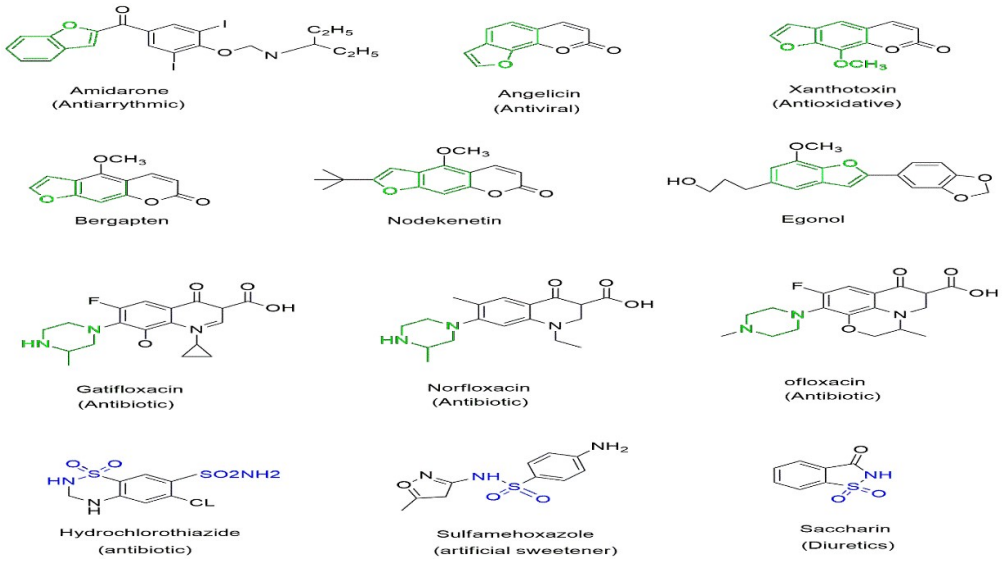


Fig 1: Benzofuran, Piperazine, and Sulfonyl contain some pharmacologically important compounds.

2. Experimental

2.1 Chemistry

All chemicals and solvents were of good analytical grade. Solvents of the analytical grade were used in this work exactly as they were given. All the reactions used glassware that had been oven-dried and magnetic stirring. Thin layer chromatography (TLC), employing silica gel TLC plates and a visualizing agent such as UV light, was used to track reactions. Both column chromatography employing silica gel and recrystallization in analytical grade ethanol have been used to purify reaction products. All compounds have been characterized using TLC, NMR, IR, and MS.

3.3 General Materials and Methods

3.3.1 Synthesis of 1-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)piperazine hydrochloride

Step-01 product (2 g, 1eq) was dissolved in 4 M 1,4-Dioxane: HCl (10.00 mL).The reaction mixture was stirred at rt for 8 progress of the reaction was monitored by TLC. After completion of starting material reaction mixture was concentrated under vacuum to dryness to afford 1.85 g off white solid. The obtained crude material was triturated with hexane (25.00 mL) to afford 1.65g final product. Nature of product: off white solid.

3.3.2 Synthesis of N-(4-cyanophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)piperazin-1-yl)acetamide

To a stirred solution Step-02 product (0.2 g,1eq) in DMF (2.00 mL) wad added K2CO3 (0.221 g, 3 eq) at 0°C.Then reaction mixture was stirred at rt for 5 min. Followed by addition of ethyl S.M-.B (0.14 g, 1.1eq). Then reaction mixture was stirred at 60°C for 8h. Progress of the reaction was monitored by TLC. After completion of starting material, reaction mixture was concentrated under vacuum add ice cold Water (100.00 mL) to it, there is yellow precipitation. The obtained ppt was filtered, washed with cold water (50.00 mL) and dried under high vacuum to Afford 1.30 g crude material. The obtained crude material was triturated with hexane (25.00 mL) to afford 1.10 final product. Nature of product: White solid.

2.2. Antimicrobial studies: A whole-cell growth inhibition assay was employed for antimicrobial screening. All of the microbial strains were acquired from them. All synthesized benzofuran derivatives were tested to determine their in vitro antibacterial activity. Compounds

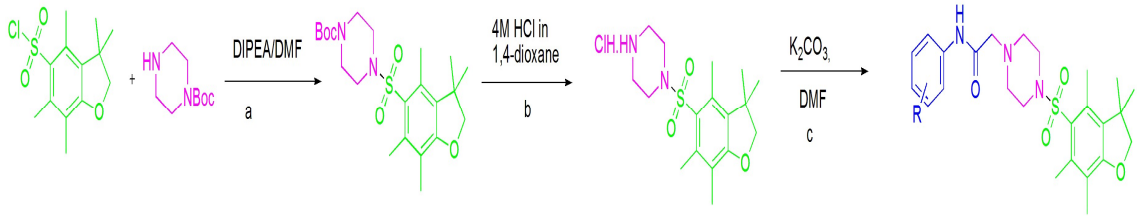
were tested using the agar diffusion method against gram-positive strains (*B.Subtilis* and *S.aureus*) and gram-negative strains (*E. coli* and *P.vulgaris*). The compounds are also tested for antifungal activity (*Aspergillus niger* and *Candida albicans*) [16].

Agar Well Diffusion Method was used to determine the antibacterial activity of the test substance. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli , Proteus vulgaris, Aspergillus niger, and Candida albicans* were each cultured individually for 24 hours. We created sterile Nutrient agar plates for bacterial cultures and sterile Chloramphenicol Yeast Glucose Agar plates for fungus cultures. A 0.2 ml culture of each type of microorganism was dispersed on various plates using sterile swabs. Four or five wells in the agar were created using an 8.0 mm cork borer on each plate. As a stock solution, a 10 mg/ml suspension of the test substance was produced in Dimethyl Sulfoxide (DMSO). Each well received 50µg/ml of the stock solution.

3. Results and Discussion

3.1 Chemistry (scheme and substitution)

The antimicrobial assessment of synthetic N-(3,5-fluorophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)piperazin-1-yl)acetamide derivatives is reported in the current work for the first time. The physical and analytical properties of the synthesized derivatives were verified utilizing methods like TLC, IR, NMR, and MS. Finally, these compounds' in vitro antibacterial and antifungal activity was examined.



SCHEME 1: Synthesis of N-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydro benzofuran-5-yl) sulfonyl)piperazin-1-yl)acetamide.

Table 1: Synthesized derivatives.

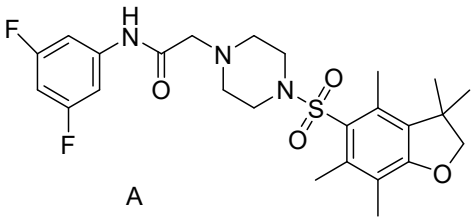
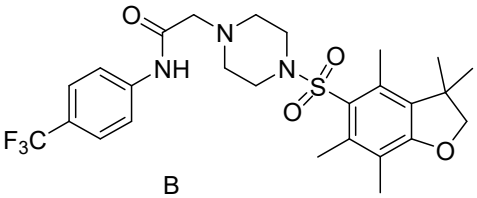
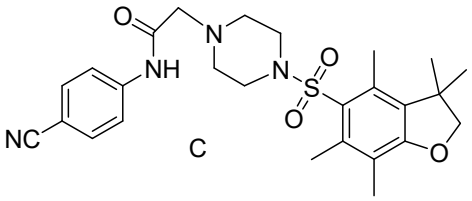
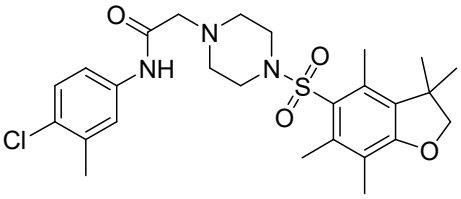
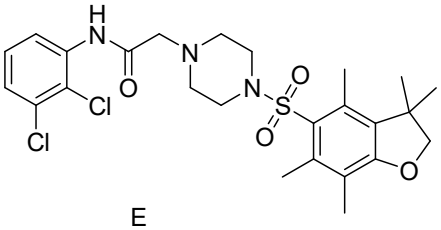
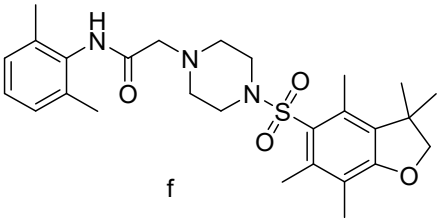
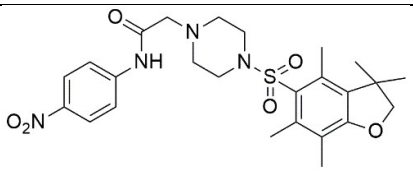
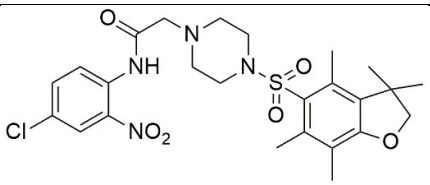
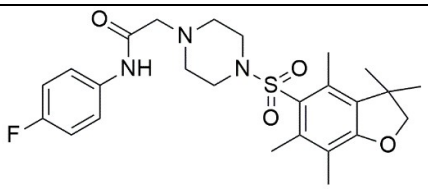
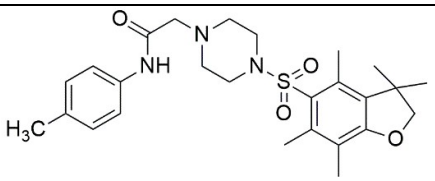
 A	 B
 C	 D
 E	 f
 G	 H
 I	 J

Table 2: Physico-chemical parameters of synthesized compounds.

Compound	R1	Molecular Formula	Molecular Weight	Melting Point	Appearance	Retention Factor	Solubility	% yield
A	3,5-(F)2	$C_{25}H_{31}F_2N_3OS_4$	507.59	304	Yellow solid	0.65	Ethanol	65.04
B	4-CF3	$C_{25}H_{32}F_3N_3OS_4$	525.58	298	Yellow-orange solid	0.70	Ethanol	67.06
C	4-CN	$C_{26}H_{32}N_40S_4$	496.62	265	White solid	0.63	Ethanol	69.12
D	4-CL, 5-CH3	$C_{26}H_{34}ClN_3OS4$	520.08	328	White solid	0.68	Ethanol	70.09
E	2,3-(CL)2	$C_{25}H_{31}CL_2N_3OS_4$	540.50	337	Brown solid	0.72	DMF	66.05
F	2-C2H5, 6-CH3	$C_{27}H_{37}N_3OS_4$	499.66	289	White solid	0.64	Ethanol	62.12
G	4-NO2	$C_{25}H_{32}N_4OS_6$	516.60	317	White solid	0.66	Ethanol	71.07
H	2-NO2, 4-CL	$C_{25}H_{31}CLN_4OS_6$	551.05	368	White solid	0.76	DMF	66.82
I	4-F	$C_{25}H_{32}FN_3OS_4$	625.73	377	Yellow solid	0.79	Ethanol	68.13
J	4-CH3	$C_{26}H_{35}N_3OS_4$	485.63	256	White solid	0.61	Ethanol	70.12

All melting points are uncorrected and were determined on a Veego melting point apparatus.

N-(3,5-difluorophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)piperazin-1-yl)acetamide (A)

% Yield: 65.04; MW: 507.59; MF: C25H31F2 N3O4S; MP: 304°C; Rf: 0.75

1H NMR: 1.44 (6H, S), 2.06 (3H,S), 2.41-2.45 (5H,dd), 2.51-2.56 (4H,dd), 3.03(2H, S), 3.11(4H, dd), 3.21-3.34(2H,S), 7.66-7.69 (2H, d), 7.85-7.88 (2H, d), 10.06(1H,S)

IR (KBr): 533, 680, 1039, 1405, 2923 MS: m/z = 508.20 [M+1]:

2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)piperazin-1-yl)-N-(4-(trifluoromethyl) phenyl) acetamide (B)

% Yield: 67.06; MW: 525.58; MF:C25H32F3N3O4S; MP: C; Rf: 0.74

1HNMR:1.44(6H,S),2.O6(3H,S),2.41,2.50(12H,dd),3.03(2H,S),3.11,3.19(6H,dd),3.34(2H,S), 6.89-6.95(1H,t),7.43-7.45(2H,dd),10.04(1H,S). IR: 534,687, 1079,1430 ,2966 ; MS: m/z: 526.21 [M+1]:

N-(4-cyanophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl) sulfonyl) piperazin-1-yl) acetamide (C)

% Yield: 69.12; MW: 496.62; MF: C26H32N4O4S; MP: 134-138°C; Rf: 0.74

1HNMR:1.44(6H,S),2.06(3H,S),2.412.45(11H,dd),2.73(1H,S),3.03(1H,S),3.10(2H,S),3.22(4 H,S),3.34(2H,S),7.76,7.86(5H,dd),10.14(1H,S).IR: 534,660, 1039 , 1405 , 1584,2923; MS: m/z: 497. 21 [M+1]:

N-(4-chloro-3-methylphenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) piperazin-1-yl) acetamide (D)

% Yield: 70.09; MW: 520.08; MF: C26H34CLN3O4S;MP: 132-207°C; Rf: 0.74

1HNMR:1.44(6H,S),2.O6(3H,S),2.29(3H,S),2.412.51(11H,dd),3.03(2H,dd),3.10(4H,dd),3.16 -3.14(2H,S),7.31(1H,S),7.34(1H,S),7.51-7.62(1H,t),9.74(1H,S). IR: 767, 1058, 1422, 1606, 2965 ; MS: m/z: 521. 08 [M+1]

N-(2,3-dichlorophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl) sulfonyl) piperazin-1-yl) acetamide (E)

% Yield: 66.05; MW: 440.50; MFC26H34CL2N3O4S: MP: 337°C; Rf: 0.70

1HNMR:1.44(6H,S)2.06(3H,S),2.42-2.50(8H,dd),2.62(4H,S),3.03(3H,S),3.12-3.24(4H,dd), 3.34(2H,S),7.35-7.44(2H,dd),8.13-8.16(1H,dd),9.93(1H,S).IR 550,765,1047,1560,1445; MS m/z: 539.14[M+1]

N-(2-ethyl-6-methylphenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) piperazin-1-yl) acetamide (F)

% Yield: 62.12; MW: 499.66; MF: C27H37N3O4S; MP: 182°C; Rf: 0.74

1HNMR:1.04-1.09(3H,S),1.44(6H,S),2.06-2.11(6H,S),2.41-2.60(13H,dd),3.03(2H,S),3.13-3.34(6H,dd),7.08-7.10(3H,dd),9.25(1H,S).IR 604,668,1050,1559,2990; MS m/z: 513.27 [M+1]

N-(4-nitrophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) piperazin-1-yl) acetamide (G)

% Yield: 71.07; MW: 516.60; MF: C25H32N4O6S; MP: 182°C; Rf: 0.74

IR 590,648,1050,1435,1548; MS m/z: 516.20[M+1]

N-(4-chloro-2-nitrophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) piperazin-1-yl) acetamide (H)

% Yield: 66.82; MW: 551.05; MF: C25H31CLN4O6S; MP: -302°C; Rf: 0.74

IR 765,1067 ,1440,1578,2960 ; MS m/z 550.16[M+1]

N-(4-fluorophenyl)-2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) piperazin-1-yl) acetamide (I)

% Yield: 70.26; MW: 499.52; MF: C27H25N5O5; MP: 182°C; Rf: 0.74

IR 540,678,1058,1430,2935; MS m/z 490.21[M+1]

2-(4-((3,3,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl) sulfonyl) piperazin-1-yl)-N-(p-tolyl) acetamide (J)

% Yield: 70.26; MW: 499.52; MF: C27H25N5O5; MP: 182°C; Rf : 0.74

IR 530,638,1064,1432,2940 ; MS m/z 485.23[M+1]

3.2 Antimicrobial activity

Ten benzofuran derivatives were examined for their in vitro antifungal and antibacterial activity in the current study.

For the antibacterial and antifungal trials, the positive control standards were taken to be Amoxicillin and Fluconazole, respectively. All chemicals have a predefined concentration of 10 g/mL for antimicrobial activity. Results of summarised antimicrobial studies were compiled.

Table 3: Antibacterial And Antifungal Activity Of Synthesized Compounds. (Minimum Inhibiting Concentration)

SR.NO	***GM +*** ***B. SUBTILIS***	***GM+*** ***S. AUREUS***	***GM-*** ***E.COLI***	***GM-*** ***P.VULGARIS***	***FUNGAL*** ***C.ALBICANS***	***FUNGAL*** ***A.NIGER***
A	21	18	16	19	18	19
B	20	15	14	17	16	20
C	18	17	11	14	14	17
D	9	12	8	16	12	13
E	7	11	10	12	9	10
F	13	9	12	9	10	14
G	NA	4	NA	7	6	7
I	3	NA	NA	NA	3	4
J	NA	NA	6	3	NA	NA
K	6	2	2	NA	NA	NA
Amoxicillin	22	20	22	20	-	-
Fluconazole	-	-	-	-	19	22

Additionally, we tested the antibacterial efficacy of recently created antifungal compounds. In comparison to the conventional medicine Amoxicillin, the tested derivatives demonstrated an up to 22 mm zone of inhibition in a 10 mg/mL concentration against Gram-negative and Gram-positive bacteria. The synthetic chemicals' antifungal efficacy was incredibly meagre.

Evaluation of anti-microbial activity.

The antibacterial activity of each of the recently developed benzofuran derivatives and reference drugs was evaluated in vitro against both gram-positive and gram-negative bacteria. Using the inhibition zone approach and minimum inhibitory concentrations (MIC), antifungal activity against *Candida albicans and A. Niger* was examined. Fluconazole was regarded as the primary antifungal drug, with Amoxicillin serving as the antibacterial drug. The results of synthesized compounds are summarized in Table 3. With the exception of G, I, J, and K, the majority of the compounds tested had a moderate to high antibacterial impact on the gram-positive bacteria *Staphylococcus aureus and Bacillus subtilis* as well as the gram-negative bacteria *Escherichia coli and P. Vulgaris*. With the exception of G, I, J, and K all synthetic compounds showed antifungal activity against *A. Niger and Candida albicans*. Compound A had superior antibacterial and antifungal action against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, P. Vulgaris, A. Niger, and Candida albicans* when compared to all other benzofuran derivatives investigated

4. Conclusion

We have described convenient methods for the synthesis of novel antimicrobial agents. Spectral and analytical data have characterized ten compounds. The in vitro antimicrobial activities of the target compounds were examined using the agar well diffusion method and the potentially active compounds were further examined using the MIC experiment. Among all, compounds A, B, and D acting as promising broad–spectrum antimicrobial agents. Particularly, compound A was the most active and broad-spectrum one. Compound A is acting on multiple strains of bacteria and Fungi.

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Ethical statement

This work does not involve the use of humans or animals.

CRediT Author Statement

Dr. Sachin A. Dhawale- Concept, Writing original draft and Supervision. All Co-authors contributed equally.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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