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Syntheses, Characterization and Biological Evaluation of 3-Methyl-2,4-Diphenyl-1,5-Benzodiazepine Derivatives

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ABSTRACT

This study described the synthesis, spectral characterization, and antimicrobial assessment of two 3-methyl-2,4-diphenyl-1,5-benzodiazepine derivatives (Compounds 2a and 2b) that were prepared by condensing chalcones with o-phenylenediamine with the aid of a microwave. Key functional groups were confirmed by FTIR spectroscopy: Compound 2b displayed N–H (3354 cm^{-1}), aromatic C–H (2970 cm^{-1}), C–N (1272 cm^{-1}), and O–H (3210 cm^{-1}) bands, whereas Compound 2a displayed N–H (3365 cm^{-1}), aromatic C–H (2935 cm^{-1}), C–N (1248 cm^{-1}), and C–O (1000 cm^{-1}) stretches. In accordance with molecular masses of 344.45 g mol^{-1} (2a) and 330.42 g mol^{-1} (2b), mass spectral fragmentation patterns validated the suggested molecular structures. The cup-plate method was used to measure the antimicrobial activity against *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, and *Escherichia coli*. With a minimum inhibitory concentration (MIC) of 1.56 mg mL^{-1} and a minimum bactericidal concentration (MBC) of 6.25 mg mL^{-1} against *Aspergillus niger*, compound 2a demonstrated the strongest antifungal activity. Methoxy substitution increased activity, and inhibition zones varied from 12.5 to 100 mg mL^{-1}. These results show that oxygen-containing 1,5-benzodiazepines produced by microwave synthesis have the potential to be effective antimicrobials.

Keywords: 1,5-Benzodiazepine, chalcones, biological activities, acetophenone, propiophenone

INTRODUCTION

Pharmacology saw a major breakthrough with the discovery of the first benzodiazepine, chlordiazepoxide (Librium), in 1955 and the subsequent release of Valium (diazepam) in 1963 [1].

By the 1970s, benzodiazepines were widely used due to their perceived safety compared to barbiturates [2]. Their role in contemporary healthcare is still shaped by the regulatory actions and ongoing discussions that followed the 1980s emergence of concerns about abuse and dependence, especially with regard to their use in elderly patients and other unforeseen challenges [1, 2].

O-phenylenediamine (OPDA) is condensed with different carbonyl compounds, like ketones or α,β-unsaturated carbonyl compounds, to create 1,5-benzodiazepines. In order to increase the effectiveness of these reactions, acidic catalysts are essential. Solid acid catalysts such as H-MCM-22, for example, have been used in recent research and greatly increase the yields of the intended products [3]. Furthermore, techniques for microwave-assisted synthesis have been investigated, which enable the quick and effective synthesis of these derivatives [3].

Studies have shown that 1,5-benzodiazepine derivatives have promising biological activities. For instance, when paired with well-known chemotherapeutics like methotrexate, some derivatives have shown synergistic effects and anticancer properties. This demonstrates their potential as complementary therapies for the treatment of cancer [4]. Additionally, research has shown that certain 1,5-benzodiazepines have antimicrobial qualities, indicating their potential for treating infections [3, 4].

The purpose of this study is to synthesize, describe, and assess the antimicrobial properties of derivatives of 3-methyl-2,4-diphenyl-1,5-benzodiazepines. The study specifically entails the synthesis of these derivatives, the structural characterization of these derivatives using FTIR and GC-MS techniques, and the evaluation of their antimicrobial potential. As far as we are aware, no prior research has documented the synthesis, biological assessment, or characterization of benzodiazepines with a methyl group at position three. Therefore, the goal of this work is to create and study these target molecules as possible novel antimicrobial agents with enhanced effectiveness and fewer adverse effects. Despite their significance in biology and pharmacology, benzodiazepines are associated with serious side effects, especially during withdrawal, and can be dangerous when taken with other depressants [2].

MATERIALS AND METHOD

Materials/ reagents

All chemicals and solvents were of analytical or reagent grade and used as received without further purification. Benzaldehyde (≥99%, Sigma-Aldrich), 4′-Hydroxypropiophenone (≥98%, Sigma-

Aldrich), 4′-Methoxypropiophenone (≥98%, Sigma-Aldrich), o-Phenylenediamine (OPD, ≥99%, Merck), piperidine (≥99%, Merck), glacial acetic acid (≥99.7%, BDH), ethanol (absolute, 99.9%, Sigma-Aldrich) and macrowave 300 W were used in the synthesis.

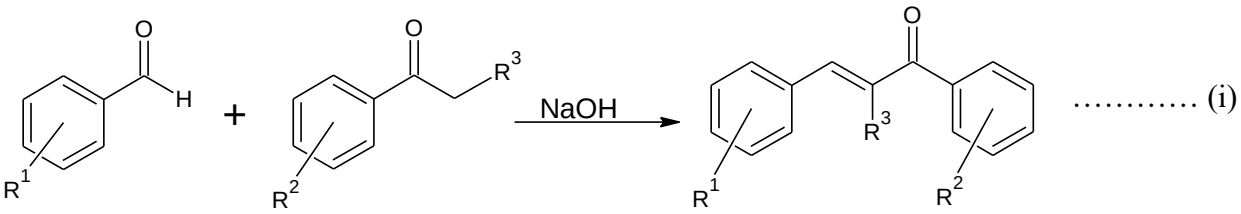
General procedure

Using microwave assisted method in the formation of the target molecule, it involved two steps; the first step is the formation of an appropriate chalcone and the second step is the cyclization reaction to form the benzodiazepines.

Syntheses of chalcones

The synthesis was carried out using the microwave irradiation techniques. Approximately 10 mL of distilled water were used to dissolve 1 g of sodium hydroxide in a 250 mL round-bottom flask. This was followed by the addition of 1.8 mL of pure benzaldehyde and 1.4 mL of the corresponding propiophenone. For 5 - 10 minutes, the mixture was exposed to 300 W of microwave radiation, shaking occasionally to guarantee even heating.

The reaction mixture was left to cool to room temperature after the irradiation was finished. After that, the thick precipitate was refrigerated for the entire night in order to finish crystallizing. A funnel and filter paper was used to filter the solid product, and distilled water was used to wash the filtrate until it was litmus-neutral. Ethanol (5 mL per gram of product) was used for recrystallization. After that, the yield of purified chalcone derivatives (1a and 1b) were computed [5, 6]. The reaction is given as equation (i) in scheme 1; Table 1 shows the confirmed structures of the synthesized chalcones and their percentages yields.



..... (i)

(1a, 1b)

R¹ = H **R³** = CH_3

R² = H, 4-OCH_3, 4-OH

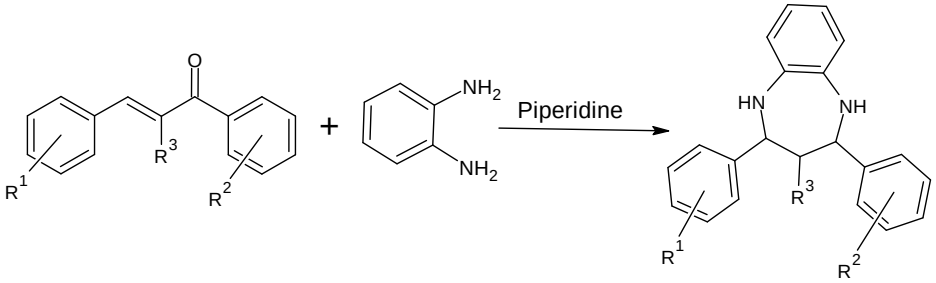
Scheme 1: Syntheses of Chalcones [5, 6]

Syntheses of benzodiazepines from chalcones

The synthesis was carried out using the microwave irradiation techniques. After dissolving chalcone derivatives (1a and 1b) (0.602 g, 2 mmol) in 30 mL of ethanol, o-phenylenediamine (0.21 g, 2 mmol) and a few drops of piperidine were added. After that, the reaction mixture was microwave-irradiated at 300 W for 5 to 10 minutes while being stirred periodically.

And 1 mL of acetic acid was added following the initial reaction, and the mixture was then exposed to radiation for 5 to 10 more minutes under the same circumstances. About half of the solvent was extracted under low pressure after microwave treatment. To enable crystallization, the resulting oily residue was allowed to stand at room temperature for the entire night.

Filtration was used to collect the solid product, which was then cleaned with cold aqueous ethanol (5 mL) and dried. Following standard procedures, the end product was obtained, after that, the yield of purified benzodiazepine derivatives (2a and 2b), was computed and characterized [7, 8]. The reaction is given as equation (ii) in scheme 2; Table 1 shows the confirmed structures of the synthesized benzodiazepines and their percentages yields.



…….. (ii)

(2a, 2b)

R¹ = H **R³** = CH_3

R² = H, 4-OCH_3, 4-OH

Scheme 2: Syntheses of Benzodiazepines [7, 8]

Spectroscopic analysis

The synthesized compounds, both the chalcones and the benzodiazepine were characterized using FT-IR [9, 10] and GC-MS [11, 12] spectroscopic methods of analyses.

Biological studies

The synthesized compounds, both the chalcones and the benzodiazepine were investigated for antimicrobial properties, on some selected clinical isolates, which include bacterial and fungal

strains. Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and fungi (*Aspergillus niger* and *Candida albicans*) and it revealed their zones of inhibition, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/ Fugacidal Concentration (MBC/ MFC) [13].

RESULTS AND DISCUSSION

Spectroscopic analyses

Identification of chalcones

(1-(4-methoxyphenyl)-2-methyl-3-phenylprop-2-en-1-one) (1a) with the molecular weight 252.31 g mol^{-1}, GC retention time 7 min and molecular formula $C_{17}H_{16}O_2$ was obtained as yellow solid powder at 88 % yield. The IR spectrum (Figure 1) showed the following prominent peaks (cm^{-1}); 2974 (C–H), 1643 (C=O), 1556 (C=C) and 1084 (C–O). The major ion signals in GC-MS (Figure 2) were: m/z 277, 252, 235 and 216. But the only fragment ion close to the molecular weight of the compound is m/z 252 peak which supported the structural confirmation.

(1-(4-hydroxyphenyl)-2-methyl-3-phenylprop-2-en-1-one) (1b) with a molecular weight of 238.28 g mol^{-1}, a GC retention time of 18 minutes, and the chemical formula $C_{16}H_{14}O_2$, Compound 1b was produced as an orange solid powder with a 51% yield. The following strong peaks (cm^{-1}) were visible in the IR spectra (Figure 3): 2974 (C–H), 1643 (C=O), 1556 (C=C), 1084 (C–O), and 3255 (O–H). In GC-MS (Figure 4), the main ion signals were m/z 284, 239, and 222. However, the m/z 239 peak, which confirmed the structural confirmation, is the only fragment ion that is near to the compound's molecular weight.

Identification of benzodiazepines

(2-(4-methoxyphenyl)-3-methyl-4-phenyl-1,5-benzodiazepine) (2a) with a molecular weight of 344.45 g mol^{-1}, a GC retention time of 32 minutes, and the chemical formula $C_{23}H_{24}N_2O$, Compound 2a was produced as a dark yellow solid powder at a 74% yield. The following notable peaks (cm^{-1}) were observed in the infrared spectrum (Figure 5): 3365 (N–H), 2935 (C–H), 1248 (C–N), and 1000 (C–O). In GC-MS (Figure 6), the primary ion signals were m/z 405, 355, 341, and 281. However, the m/z 341 peak, which indicated the structural confirmation, is the only fragment ion that is very close to the compound's molecular weight.

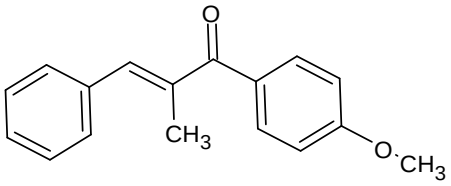
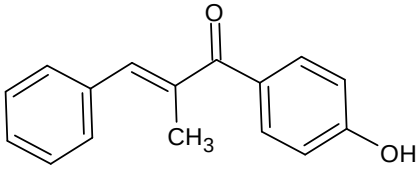
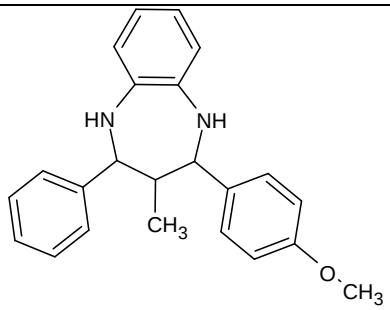
(4-(3-methyl-4-phenyl-1,5-benzodiazepin-2-yl)phenol) (2b) with a molecular weight of 330.42 g mol^{-1}, a GC retention time of 25 minutes and the chemical formula $C_{22}H_{22}N_2O$, Compound 2b

was produced as a light yellow solid powder with an 81% yield. The most significant peaks (cm^{-1}) in the infrared spectrum (Figure 7) were 3354 (N–H), 2970 (C–H), 1272 (C–N), and 3210 (O–H). In GC-MS, the main ion signals were m/z 405, 355, 327, and 281 (Figure 8). However, the m/z 327 peak, which confirmed the structural confirmation, is the only fragment ion which falls closest to the compound's molecular weight.

Biological studies

The results for the zones of inhibition (ZOI) (Table 3), Minimum Inhibitory Concentration (MIC) (Table 4) and Minimum Bactericidal/ Fugacidal Concentration (MBC/ MFC) (Table 5) of the synthesized target molecules are shown below.

Table 1. Structural formula and percentage yield of chalcones and benzodiazepine Derivatives

Compound	Structural formula	Yield (%)
1a		88
1b		51
2a		74

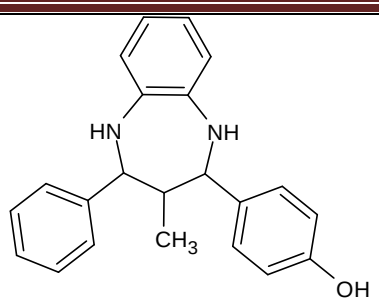
2b		81

Table 2. Retention Times, Electron Impact Ions and Relative Intensity of chalcones and benzodiazepine Derivatives

Compound	**Molecular formula**	**Molecular weight ($gmol^{-1}$)**	**Retention time (min.)**	**Mass/charge**
1a	$C_{17}H_{16}O_2$	252.31	7	277, **252**, 235 and 216
1b	$C_{16}H_{14}O_2$	238.28	18	284, **239** and 222
2a	$C_{23}H_{24}N_2O$	344.45	32	405, 355, **341** and 281
2b	$C_{22}H_{22}N_2O$	330.42	25	405, 355, **327** and 281

Table 3. Antimicrobial Activity (Zone of Inhibition mm) of 3-methyl-2,4-diphenyl-1,5-benzodiazepine Derivatives

Compound	**Concentration (mg/mL)**	**Inhibition zone (mm)**					
		E. *coli*	S. *aureus*	B. *subtilis*	P. *aeruginosa*	A. *niger*	C. *albican*
2a	100	20	18	16	19	31	23
	50	18	16	14	17	27	18
	25	15	13	12	15	18	14
	12.5	13	11	-	13	14	12
	Control drug	35	25	30	40	46	22
	100	16	15	17	14	15	19
	50	14	13	15	12	12	17

	25	12	-	13	-	-	13
2b	12.5	-	-	-	-	-	-
	Control drug	36	24	30	40	45	20

Control drug: Ciprofloxacin 10µg/mL for bacteria; Terbinafine 30µg/mL for fungi

Table 4. Minimum Inhibitory Concentrations of the Synthesized Compound from Chalcones Against the Organisms

Microorganism	**Compound (MIC)**	
	2a (mg/mL)	**2b (mg/mL)**
E. *coli*	6.25	25
S. *aureus*	12.5	50
B. *subtilis*	25	25
P. *aeruginosa*	12.5	50
A. *niger*	1.56	50
C. *albicans*	3.12	12.5

Table 5: Minimum Bactericidal/ Fungacidal Concentrations of the Synthesized Compound from Chalcones Against the Organisms

Microorganism	**Compound (MBC/ MFC)**	
	2a (mg/mL)	**2b (mg/mL)**
E. *coli*	25	50
S. *aureus*	25	100
B. *subtilis*	50	50
P. *aeruginosa*	25	100
A. *niger*	6.25	100
C. *albicans*	12.5	25

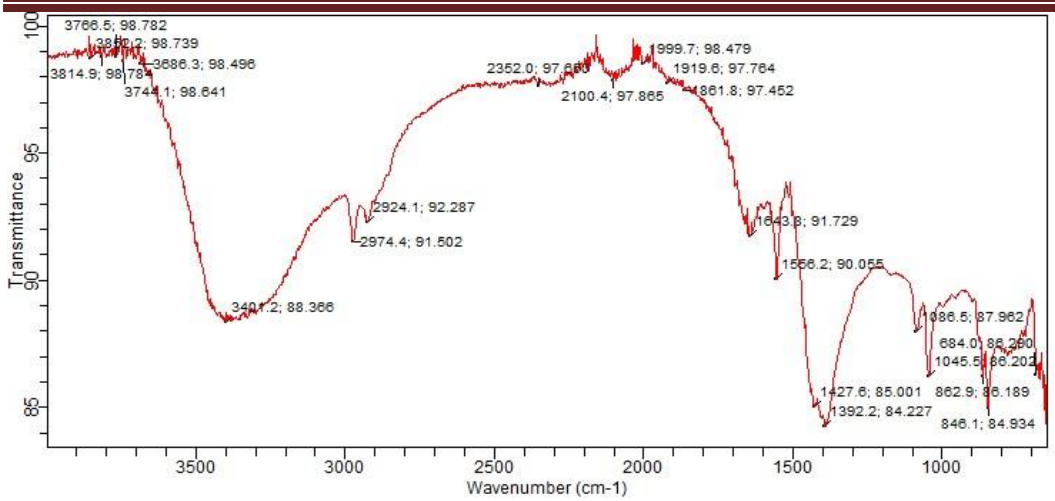


Figure 1: FT-IR spectrum of Compound 1a: (1-(4-methoxyphenyl)-2-methyl-3-phenylprop-2-en-1-one)

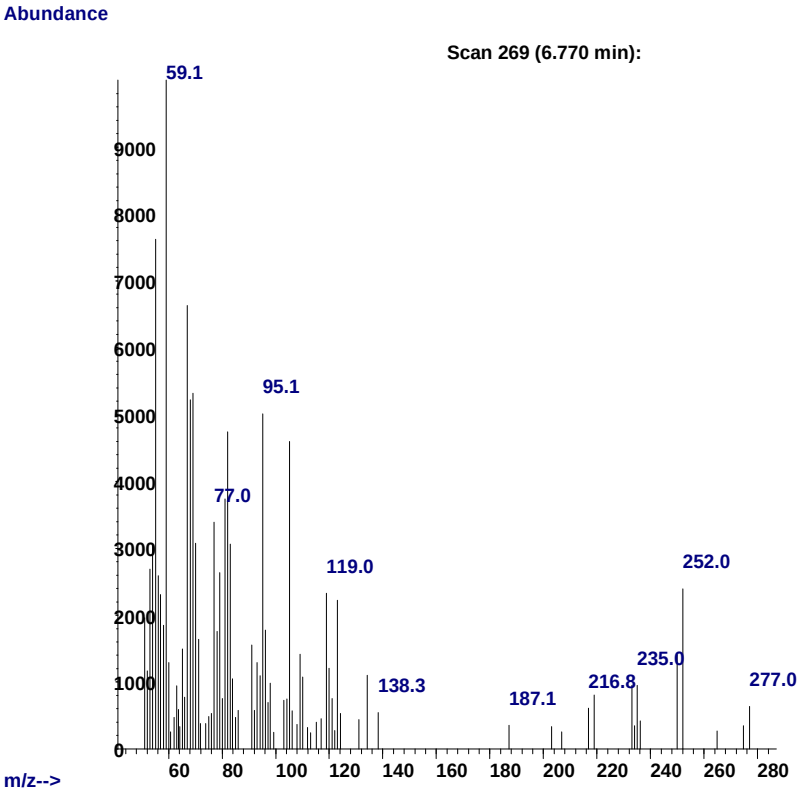


Figure 2: GC-MS spectrum of Compound 1a: (1-(4-methoxyphenyl)-2-methyl-3-phenylprop-2-en-1-one) at Scan 269 (6.770 min)

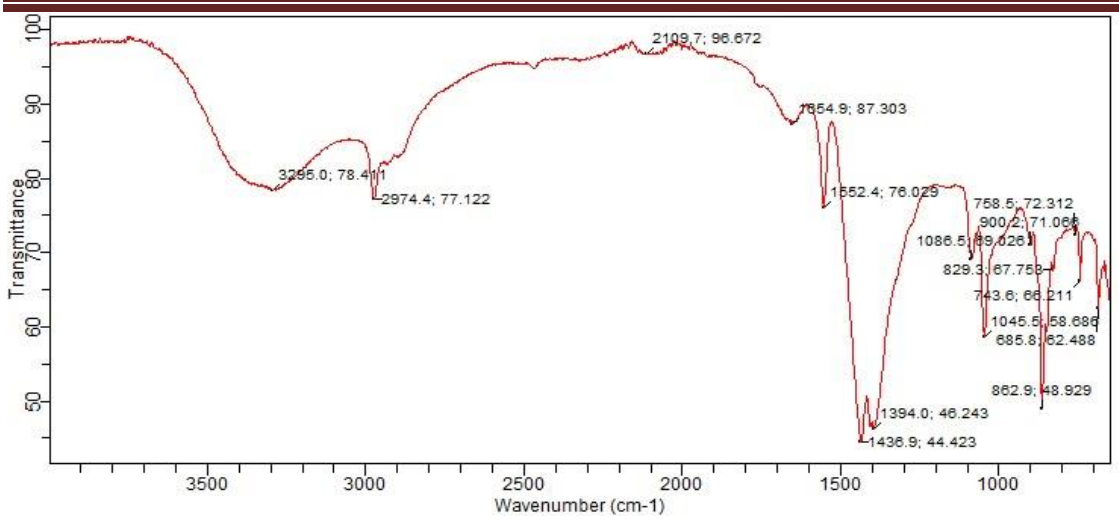


Figure 3: FT-IR spectrum of Compound 1b: (1-(4-hydroxyphenyl)-2-methyl-3-phenylprop-2-en-1-one)

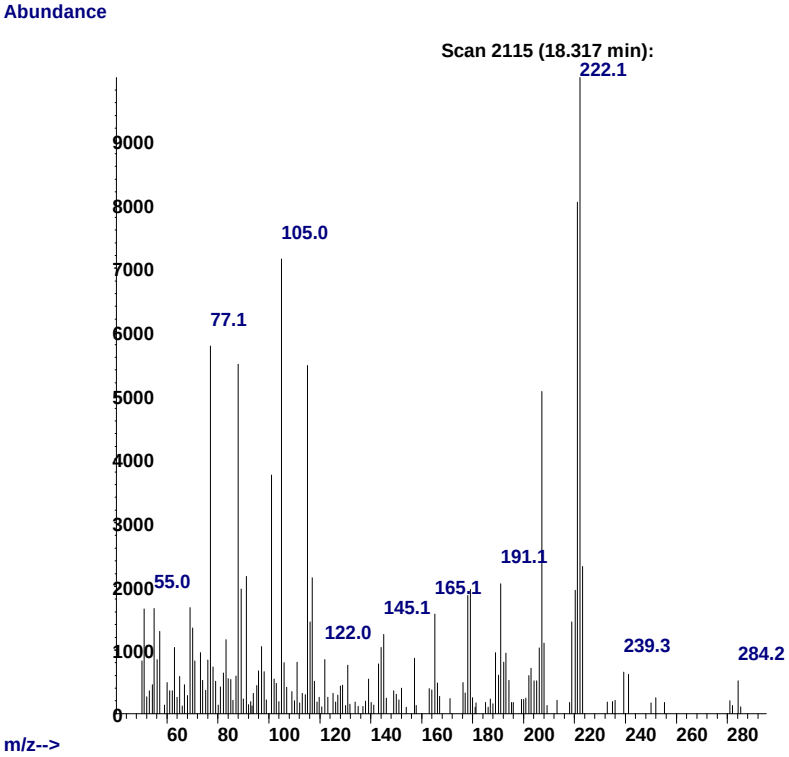


Figure 4: GC-MS spectrum of Compound 1b: (1-(4-hydroxyphenyl)-2-methyl-3-phenylprop-2-en-1-one) at Scan 2009 (17.654 min)

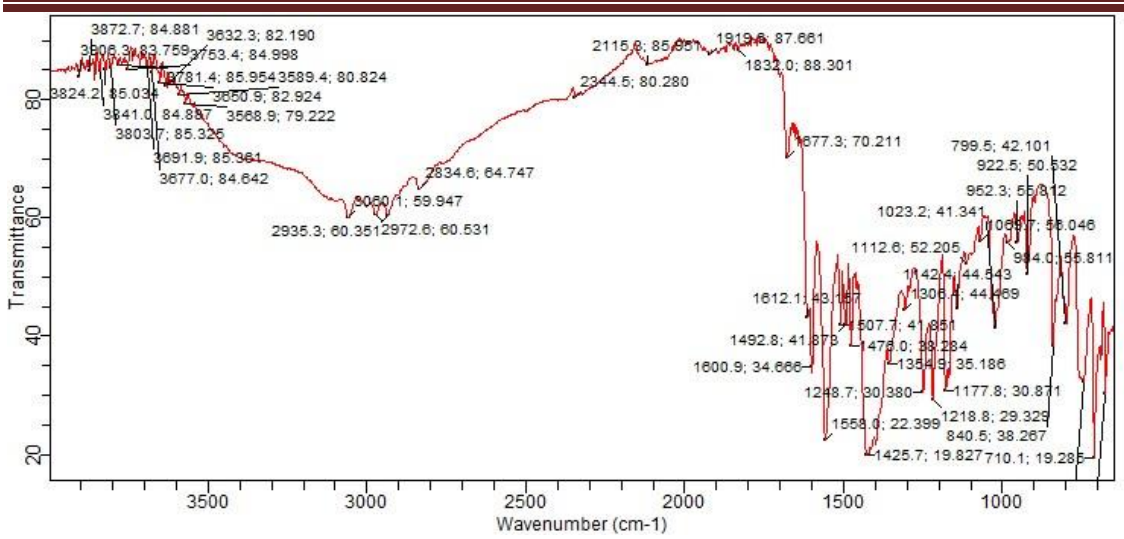


Figure 5: FT-IR spectrum of Compound 2a: (2-(4-methoxyphenyl)-3-methyl-4-phenyl-1,5-benzodiazepine)

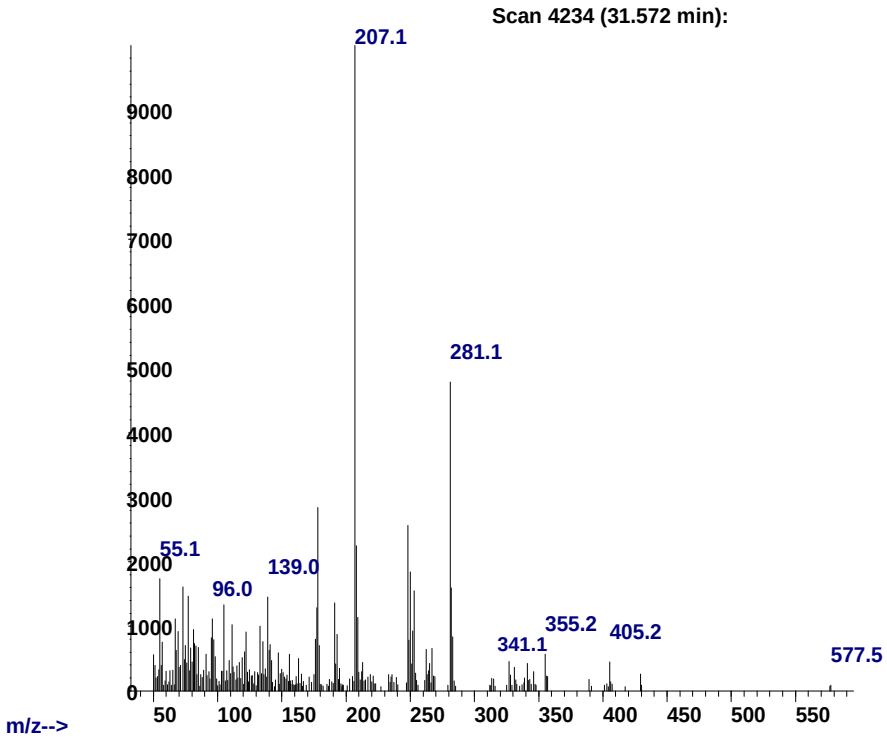


Figure 6: GC-MS spectrum of Compound 2a: (2-(4-methoxyphenyl)-3-methyl-4-phenyl-1,5-benzodiazepine) at Scan 4234 (31.572 min)

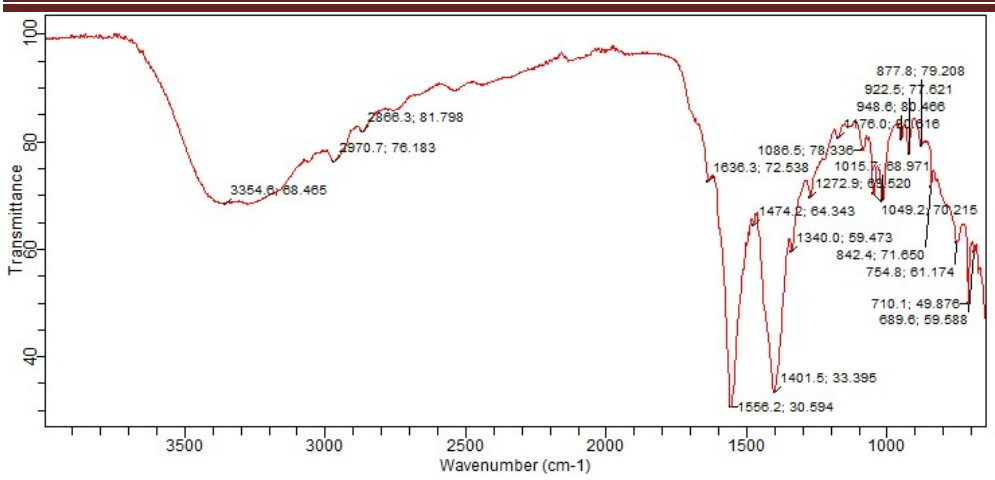


Figure 7: FT-IR spectrum of Compound 2b: (4-(3-methyl-4-phenyl-1,5-benzodiazepin-2-yl)phenol)

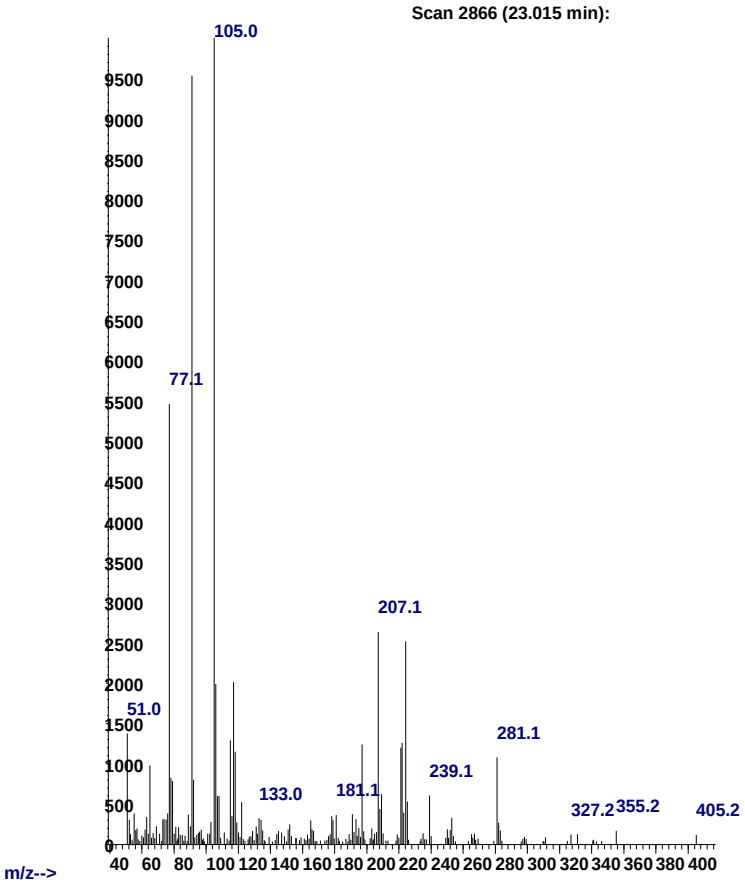


Figure 8: GC-MS spectrum of Compound 2b: (4-(3-methyl-4-phenyl-1,5-benzodiazepin-2-yl)phenol) at Scan 3237 (25.335 min)

The effectiveness of new 3-methyl-2,4-diphenyl-1,5-benzodiazepine compounds was tested against various bacteria and fungi, including E. *coli*, S. *aureus*, B. *subtilis*, P. *aeruginosa*, A. *niger*, and C. *albicans* as shown in Table 3. The study evaluated two selected derivatives labeled as Compound 2a and 2b at concentrations ranging from 12.5 to 100 mg/mL. The results were compared with a standard control drug (Ciprofloxacin 10 µg/mL for bacteria and Terbinafine 30 µg/mL for Fungi), and zones of inhibition were used to determine the extent of antimicrobial efficacy [14].

The antimicrobial activity of Compound 2a has inhibition zones at 100 mg/mL, which are 20 mm for E. *Coli*, 18 mm for S. *aureus*, 16 mm for B. *subtilis*, 19 mm for P. *aeruginosa*, 21 mm for A. *niger*, and 23 mm for C. *albicans*. Compound 2a have the highest antibacterial activity and comparatively strong antifungal activity. The antifungal properties of benzodiazepine analogs with electron-withdrawing groups [15]. The activity was significant even though it had less of an impact on P. *aeruginosa*. The derivative's structural characteristics, which permitted limited interaction with resistant Gram-negative outer membranes, may be responsible for the moderate inhibition [16, 17].

Compound 2b had a little less effectiveness than Compound 2a, but the results were comparable. It showed inhibition zones of 16 mm for E. *Coli*, 15 mm for S. *aureus*, 17 mm for B. *subtilis*, 14 mm for P. *aeruginosa*, 15 mm for A. *niger*, and 19 mm for C. *albicans* at 100 mg/mL. Because of its intermediate substitution or partial structural similarity to known active agents, the Compound 2b showed consistent antimicrobial activity against both Gram-positive and Gram-negative strains, but its zones of inhibition were smaller than those produced by the control medication, so it may still have moderate antimicrobial potential [16].

With inhibition zones ranging from 20 mm to 46 mm for every tested organism, the control drug demonstrated noticeably greater antimicrobial activity. This comparison demonstrates that while the synthesized derivatives, specifically Compound 2a, showed encouraging activity, their effectiveness is still lower than that of the reference standard. Significantly, the control medication demonstrated the greatest inhibition against P. *aeruginosa* (40–41 mm) and A. *niger* (45–46 mm), indicating superior Gram-negative and antifungal coverage.

The study's conclusions support the possibility that 1,5-benzodiazepine derivatives could be effective antimicrobials. Their range of activity and potency are significantly influenced by

structural changes. The findings align with recent studies that highlight the importance of benzodiazepine scaffolds in drug discovery [18, 19, 20].

The lowest concentration of an antimicrobial substance needed to prevent a microorganism's visible growth following the incubation is known as the minimum inhibitory concentration, or MIC [21]. The MIC values of two synthetic 3-methyl-2,4-diphenyl-1,5-benzodiazepine derivatives (as Compound 2a and 2b) against a selection of bacterial and fungal pathogens, such as E. *coli*, S. *aureus*, B. *subtilis*, P. *aeruginosa*, A. *niger*, and C. *albicans*, are shown in Table 4.

There were significant differences between the compound and microorganisms tested, with MIC values ranging from as low as 1.56 mg/mL to as high as 50 mg/mL. Since less compound is needed to inhibit microbial growth, lower MIC values are signs of greater antimicrobial effectiveness [22].

Compound 2a demonstrated the strongest antifungal activity. Its lowest MICs were 3.12 mg/mL against C. *albicans* and 1.56 mg/mL against A. *niger*. With MICs of 6.25 mg/mL against E. *coli*, 12.5 mg/mL against S. *aureus*, and 12.5 mg/mL against P. *aeruginosa*, it also demonstrated strong antibacterial activity.

The addition of electron-withdrawing substituents, which are known to improve membrane permeability and strengthen binding to fungal enzymatic targets, may be responsible for the synthesized 1,5-benzodiazepine analogs' increased antifungal potency [15, 23].

The antimicrobial activity of Compound 2b was moderate. It found MIC values of 12.5 mg/mL against C. *albicans* and 25 mg/mL against B. *subtilis* and E. *coli*. With MICs of 50 mg/mL, it demonstrated reduced effectiveness against S. *aureus*, P. *aeruginosa*, and A. *niger*. The observed pattern points to a partial effectiveness that may be restricted by a less powerful interaction with microbial targets [24]. Despite being less effective than Compound 2a, Compound 2b's moderate activity indicates that it still has some antimicrobial potential and might benefit from improving its structure to increase its activity [25, 26].

Based on a comparison of MIC values, Compound 2a was the strongest overall, demonstrating the strongest antifungal activity against A. *niger* (1.56 mg/mL). The concentrations used for control drug for Bacteria (Ciprofloxacin) is 10 µg/mL and fungi (Terbinafine) is 30 µg/mL. The benzodiazepine ring modifications, particularly those involving electron-

withdrawing groups, halogenation, or ring conjugation, can greatly increase antimicrobial efficacy by enhancing membrane permeability and enzyme binding affinity [18, 23].

The lowest concentration of an antimicrobial agent needed to eradicate a particular microorganism is known as the Minimum Bactericidal/ Fungacidal Concentration (MBC) [27, 28].

Table 5 displays the MBC/ MFC values of the synthetic 3-methyl-2,4-diphenyl-1,5-benzodiazepine derivatives (Compound 2a and 2b) against specific microorganisms. These include C. *albicans*, P. *aeruginosa*, B. *subtilis*, S. *aureus*, E. *coli*, and A. *niger*.

The compounds' MBC/ MFC values varied from 6.25 mg/mL to 100 mg/mL, as indicated in Table 5, suggesting a range of bactericidal and fungicidal activities across the test organisms. The antimicrobial effectiveness of the synthesized compounds is revealed by the comparing the MBC values, which shows significant differences depending on the microbial strain and compound structure [22, 26].

With the lowest MBC value of 6.25 mg/mL, Compound 2a demonstrated impressive fungicidal activity against A. *niger*. Additionally, it demonstrated strong killing potential against various microbial classes by performing effectively against P. *aeruginosa* (25 mg/mL) and C. *albicans* (12.5 mg/mL). The comparatively lower activity against certain Gram-positive bacteria is indicated by the higher MBC value against B. *subtilis* (50 mg/mL). With better results against B. *subtilis* (50 mg/mL) and C. *albicans* (25 mg/mL), Compound 2b demonstrated moderate activity. However, S. *aureus*, P. *aeruginosa*, and A. *niger* still required higher concentrations (100 mg/mL), suggesting limited but partial effectiveness. The concentrations used for control drug for bacteria (Ciprofloxacin) is 10 µg/mL and fungi (Terbinafine) is 30 µg/mL.

CONCLUSION

Using a microwave-assisted technique prove a more effective and more rapid methods. The Compound 2a and 2b has the percentage yield 74 and 81 % respectively. FTIR analysis identify the following functional groups N-H, C-H, C-N, C-O and OH bond which are consistent with the compounds formation and their respective substituents.

GC-MS analysis of the target molecules identify the following m/z peak 355, 341, 330 and 327 which are closely corresponding to the theoretical molecular weights.

The compounds were confirmed to have an effective antibacterial and antifungal activity. According to MIC and MBC values, Compound 2a continuously demonstrated the highest antimicrobial activity and wide ranging effectiveness. Compound 2b exhibited moderate

antimicrobial activity, whereas Compound 2a exhibited the strongest antifungal activity, with the lowest MIC value. It clearly shows that substituents influence the antimicrobial activity of the compounds.

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