

Original Research Article

Antioxidant, biofilm inhibition and mutagenic activities of newly substituted fibrates

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Abstract

Purpose: A series of benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazides (**1-10**) were synthesized and assayed for their biofilm inhibition, antioxidant and mutagenic activities.

Methods: All derivatives were prepared by condensation of various substituted benzaldehyde and acetophenones with 2-(4-bromophenoxy)-2-methyl propane hydrazide, which was itself prepared by hydrazinolysis of ethyl-2-(4-bromophenoxy)-2-methyl propanoate and were characterized by FTIR, ¹H NMR, ¹³C NMR, mass spectrometry. They were screened for their in-vitro anti-oxidant, biofilm inhibition and mutagenicity by established methods.

Results: Anti-oxidant results revealed that the electron donating group enhanced the scavenging ability of the compounds as seen in compounds **4b**, **4h** and **4i**. In biofilm inhibition studies, all compounds were more active against Gram -ive bacterial strain when compared to gram +ive strain. The mutagenicity assay results indicate that the compound having chloro group substitution is mutagenic.

Conclusion: The benzylidene compounds of 2-(4-bromophenoxy)-2-methyl hydrazide possessing electron donating substituents exhibit superior activities to the electron withdrawing group substituents.

Keywords: Fibrate derivatives, Anti-oxidant, Biofilm inhibition, Mutagenic activity

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INTRODUCTION

Fibrates are a class of drugs having a stable phenoxy isobutyric acid (pharmacophore moiety) [1] used for the treatment of coronary heart disease, ischemic cerebrovascular diseases and peripheral vascular disorder [2]. Furthermore, these fibrates inhibit the accumulation of lipids in arteries and stimulate the production of high density lipoprotein cholesterol [3]. Guido Bargellini introduced the synthesis of fibric acid compound [4] and found it very responding to

human bio-chemical system for the treatment of dyslipidemia. Later, many other fibrates like Bezafibrate, Clofibrate, Fenofibrate and Ciprofibrate [5] were reported. In the last decade, scientists have coupled this moiety with various heterocyclic nuclei such as benzoxazole [6], indole-fibrate [7], amide-fibrate [8], tetrazole [9] and oxadizole [10] to get more potent biologically active compound.

Keeping in view the stable structure of fibrates, their tremendous biological activities and

absence of disproportionation, the ethyl 2-(4-bromophenoxy)-2-methyl propanoate having fibrate moiety was synthesized and converted into biologically active azomethine compounds and investigated their antioxidant, biofilm inhibition and mutagenic properties with respect to the presence substituents and azomethine linkage.

EXPERIMENTAL

Chemicals, reagents and equipment

All the chemicals were purchased from E. Merck, Sigma Aldrich and Fluka and were used without purification. Bruker Tensor 27 spectrophotometer used for IR spectra (in KBr disk). For ^1H NMR Bruker Avance-III instrument was used. TMS (tri-methyl silane) was used as an internal standard and chemical shifts were reported in ppm. For elemental analysis Perkin Elmer 2400-CHNS Analyzer was used, while Melting points were record on a Stuart scientific SMP3 apparatus and are uncorrected.

General procedure for the synthesis of *N*'-Benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazides (5a-5j)

For the synthesis of azomathine derivatives, using a modified literature method 2-(4-Bromophenoxy)-2-methyl propanonic acid (**1**) and ethyl-2-(4-bromophenoxy)-2-methyl propanoate (**2**) were synthesized [11]. Ethyl-2-(4-bromophenoxy)-2-methyl propanoate (**2**) was converted into 2-(4-bromophenoxy)-2-methyl hydrazide (**3**) by refluxing ethyl-2-(4-bromophenoxy)-2-methyl propanoate (**2**) (0.01 mol) with hydrazine hydrate (0.01 mol) in the presence of ethanol (30ml) for a period of 8hrs. Completion of reaction was monitored by TLC (hexane: ethyl acetate). The contents of flask were reduced to half through distillation and cooled to room temperature. The remaining reaction mixture on constant stirring was acidified with HCl (5%) to get the product which was filtered, washed, dried and the yield of the product obtained was 80%. Aromatic aldehydes or acetophenones (0.875 mmole), ethanol (30 mL) and phosphoric acid (1-2 drops) with 2-(4-bromophenoxy)-2-methyl hydrazide (**3**) (200 mg; 0.875 mmole) were refluxed in round bottom flask for the period of 3-4 hrs. When the reaction was completed (monitored by TLC), the content of flask was cooled to room temperature and evaporation of the solvent under vacuum yields the solid product. The residue was purified by recrystallization from ethyl alcohol.

Biofilm inhibition assay

The inhibition of biofilm formation was determined following a reported procedure [12]. The wells of a sterile 96-well flat bottomed tissue culture plates were filled with nutrient broth (100 μL , Oxoid, UK), testing sample (100 μL) and bacterial suspension (20 μL) was inoculated. Negative control wells contained nutrient broth and microbial strain while positive control contained standard antibiotic Rifampicin and nutrient broth only. Tissue cultured Plates were incubated under aerobic condition for 24 h at 37°C. Thereafter, each plate were treated with buffer of pH: 7.2 (phosphate buffer) for removal of non-adherent bacteria, for remaining bacteria all plates were treated with methanol. Afterward Crystal violet (50%) was used for staining and excess stain rinsed off. The OD of each well was measured at 630 nm using micro plate reader (BioTek, USA) and bacterial growth inhibition (INH) percent was calculated as in Eq 1.

$$\text{INH (\%)} = [100 - (\text{OD}_{630\text{sample}} \times 100)] / \text{OD}_{630\text{control}} \cdot 1$$

where OD = optical density.

Antioxidant assay

The antioxidant activity of compounds was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method [13] with slight modification. 0.004 % (1 mL) of DPPH in methanol was added to sample solution (3 mL) and the mixture was kept in the dark for 30 min. The decrease in absorbance was noted at 517 nm. Ascorbic acid was taken as the standard, and inhibition (%) was calculated as in Eq 2.

$$D (\%) = (A_0 - A_1 \times 100) / A_0 \dots\dots\dots (2)$$

where A_1 = absorbance of sample; A_0 = absorbance of blank.

Mutagenic screening assay

Mutagenicity of synthesized compounds was determined by Ames bacterial reverse-mutation method [14]. A fresh strain of Salmonella culture (test strain), sample solution, standard and reagent mixture were combined in the following ratio (Table 1).

Mutant strains of *Salmonella* (TA98 and TA100) were used. The prepared contents (200 μL) was dispensed into each well of a 96-well micro titration plate and incubated at 37 °C for 4 days.

Table 1: Ratio of standards and compounds

Variable	Volume used (mL)				
	Standard	Sample	Reagent mixture	Deionized water	Test strain
Blank	-	-	2.5	17.5	-
Background	-	-	2.5	17.5	0.005
Standard Mutagen	0.1	-	2.5	17.4	0.005
(test) sample	-	0.005	2.5	17.5	0.005

The blank plate was first observed and the rest of plates were read only when all wells in the blank plate were colored purple-an indication that the assay was not contaminated. The background, standard, and test plates observed visually, all yellow, partial yellow or turbid wells were scored as positive wells while purple wells were scored as negative. For a compound to be mutagenic, the number of positive wells had to be more than twice the number of positive well in the background plate.

RESULTS

Chemistry

2-(4-Bromophenoxy)-2-methyl propane hydrazide (**3**), was synthesized by hydrazinolysis of ethyl 2-(4-bromophenoxy)-2-methyl propanoate (**2**) which was obtained by the esterification of 2-(4-bromophenoxy)-2-methyl propanoic acid (**1**) in the presence of sulfuric acid as catalyst (Figure-1). Condensation of aromatic aldehydes and acetophenones with 2-(4-bromophenoxy)-2-methyl propane hydrazide (**3**) in ethanol yields the benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazide (**4a-4j**) compounds, all the compounds were characterized by using different spectroscopic techniques (FTIR, ^1H NMR, ^{13}C NMR and mass spectrometry). Compounds (**4a-4j**) are white to pale yellow crystalline solids. They exhibit sharp melting points and are fairly soluble in ethanol and other common organic solvents.

The presence of functional groups in compounds (**4a-4j**) were established using FTIR spectroscopy by comparing their spectra with precursors. Useful information obtained are as follows; the appearance of characteristic peak of $-\text{NH}$ around 3240 cm^{-1} indicate the conversion of primary amine into secondary amine which is related to reported value of $3120 - 3330\text{ cm}^{-1}$ [15]. The absence of peak at range of $1700 - 1750\text{ cm}^{-1}$ further confirmed the conversion of aldehyde ($-\text{CHO}$) and acetophenone ($-\text{C}=\text{O}$) group into $-\text{C}=\text{N}-$ linkage [16]. Appearance of two other characteristic peaks between $1583 - 1600\text{ cm}^{-1}$ and $1715 - 1759\text{ cm}^{-1}$ can be attributed to $\text{C}=\text{N}$ and $\text{C}=\text{O}$ respectively and are closely related with the literature value of acetate

and azomethine moiety ($1590 - 1670\text{ cm}^{-1}$ and $1720 - 1750\text{ cm}^{-1}$) [17]. The presence of these functional groups NH , $\text{C}=\text{N}$ and $\text{C}=\text{O}$ agree with the proposed structures of desired derivatives.

^1H NMR spectra were recorded in $\text{DMSO}-d_6$ and the values of chemical shifts are given in the experimental section. The important peaks of $-\text{NH}$ and methyl group $-\text{C}(\text{CH}_3)_2$ protons appears at $9.68 - 11.11\text{ ppm}$ and $1.49-1.64\text{ ppm}$ respectively. A strong singlet peak of $-\text{CH}$ of the aldehyde group in **4a**, **4b**, **4c**, **4d**, **4e** and **4f** appears between $8.12 - 8.72\text{ ppm}$ [18]. Similarly, azomethine compounds synthesized from acetophenones (**4g**, **4h**, **4i** and **4j**) also shows sharp singlet peak of $-\text{CH}_3$ around $2.20 - 3.85\text{ ppm}$ which are close to literature value of 2.50 ppm [19]. The azomethine ligands shows multiplet signals around $6.88 - 7.99\text{ ppm}$ which can be assigned as protons of benzene ring. The presence of substituents on benzene ring greatly affects the chemical shift values as seen in **4h**-whereas the hydroxyl group present at the ortho position appears as a singlet at 9.64 ppm which is close to the literature value of 10.22 ppm [20]. In ^{13}C -NMR, the representative amide group peak in all compounds appears in the range of $168-155\text{ ppm}$ which matches reported value of $167 - 152\text{ ppm}$. Similarly, aromatic carbon atoms were seen between $110-150\text{ ppm}$ depending on their electronic environment and non-aromatic carbon atoms found in the methoxy group of **4b** and **4i** were observed at 55.32 ppm which correlates with reported value of 55.91 ppm [21]. The methyl group in **4g**, **4h**, **4i** and **4j** shows a sharp signal at 25.29 ppm .

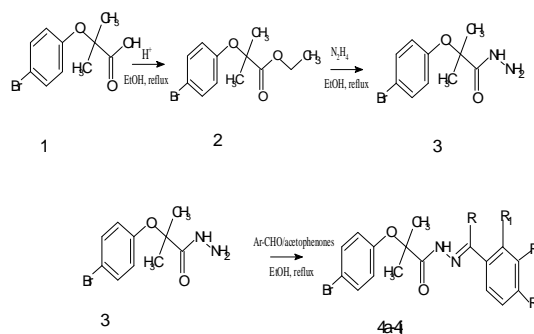


Figure 1: Synthesis of benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazide

4-Bromobenzylidene-2-(4-chlorophenoxy)-2-methylpropanehydrazide (4a)

Light yellow crystals; Yield 72 %; m.p, 176-177 °C; IR (KBr) cm^{-1} : 3241(NH), 1745(C=O), 1625(C=N); ^1H NMR (DMSO- d_6 300 MHz) δ : 9.74 (s, 1H, NH), 8.12 (s, 1H, CH), 7.76-7.68 (m, 2H, ArH) 7.41-7.37(m, 2H, ArH) 7.28 – 7.25 (m, 2H, ArH), 6.95 – 6.91 (m, 2H, ArH), 1.62 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO,100 MHz): 168.52, 152.74, 145.86, 138.22, 132.50, 131.97, 129.58, 129.05, 128.73, 120.19, 82.33, 25.29; ESI-MS m/z (%): 396.01[M+1]⁺; Anal. Calcd for C₁₇H₁₆BrClN₂O₂: C, 58.12; H, 4.59; N, 7.97; Found C, 58.09; H, 4.54; N, 8.06.

2-(4-Bromophenoxy)-2-methoxybenzylidene-2-methylpropanehydrazide (4b)

Off white powder; Yield:90 %; m.p:188-189 °C; IR (KBr) cm^{-1} : 3341(NH), 1758(C=O), 1583(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 9.64 (s, 1H, NH), 8.72 (t, $J=0.9$ HZ, 1H,CH), 7.91-7.68 (m,2H,ArH) 7.52-7.37(m, 2H, ArH) 7.28 –7.21 (m, 2H, ArH), 6.90–6.88 (m, 2H, ArH), 3.92(S, 3H, OCH₃) 1.62 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO,100 MHz): 168.52, 158.35, 152.74, 145.88, 132.50, 129.58, 128.93, 126.04, 120.19, 113.75, 82.33, 55.32, 25.29;ESI-MS m/z (%):392.1[M+1]⁺;Anal. Calcd for: C₁₈H₁₉BrN₂O₃: C, 62.32; H, 5.53; N, 8.07; Found C, 62.42; H, 5.55; N, 7.97.

2-(4-Bromophenoxy)-2-methyl-(4-nitrobenzylidene) propanehydrazide (4c)

Light yellow crystal; Yield 80 %; m.p,158-160 °C; IR (KBr) cm^{-1} : 3424(NH), 1748(C=O), 1598(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 9.95 (s, 1H, NH), 8.37 (s, 1H,CH), 8.26 (d, $J=9.0$ Hz,2H, ArH),7.92(d, $J=8.9$ Hz 2H, ArH) 7.30 – 7.26 (m, 2H, ArH), 6.94 – 6.92 (m, 2H, ArH), 1.63 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO,100 MHz): 168.52, 152.74, 147.43, 145.73, 138.88, 132.50, 129.58, 127.93, 123.65, 120.19, 82.33, 25.29; ESI-MS m/z (%): 407.1[M+1]⁺; Anal. Calcd for: C₁₇H₁₆BrN₃O₄: C, 56.44; H, 4.49; N, 11.61; Found C, 56.49; H, 4.55; N, 11.67.

3-Chlorobenzylidene-2-(4-bromophenoxy)-2-methylpropanehydrazide (4d)

Off white crystal; Yield 88%; m.p, 152-154 °C; IR (KBr) cm^{-1} : 3236(NH), 1725(C=O), 1588(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 9.80 (s, 1H, NH), 8.18(s, 1H,CH) 7.79 (t, $J=0.9$ Hz, 1H, ArH), 7.61-7.58 (m, 1H, ArH), 7.40 – 7.25 (m, 4H, ArH), 7.02 – 6.96 (m, 2H, ArH), 1.59 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO,100 MHz): 168.52, 152.74, 146.25, 134.28, 133.01, 132.50, 131.26, 129.58, 128.28, 126.49, 124.18,

120.19, 82.33, 25.29; ESI-MS m/z (%):395.6[M+1]⁺; Anal. Calcd for: C₁₇H₁₆BrClN₂O₂: C, 58.13; H, 4.59; N, 7.98; Found C, 58.12; H, 4.56; N, 8.01.

2-(4-Bromophenoxy)-2-methyl-(3-nitrobenzylidene) propanehydrazide (4e)

Pale yellow powder; Yield 87%; m.p:175-177 °C;IR (KBr) cm^{-1} : 3041(NH), 1723(C=O), 1600(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 10.11 (s, 1H,NH), 8.46 (td, $J=1.8, 1.1$ Hz, 1H,CH), 8.12 – 8.01 (m, 1H, ArH), 8.01 – 7.90 (m, 2H, ArH), 7.78 (t, $J=7.8$ Hz, 1H, ArH), 7.40 – 7.29 (m, 2H, ArH), 6.85–6.74 (m, 2H, ArH), 1.52 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO,100 MHz): 168.52, 152.74, 147.43, 145.73, 138.88, 132.50, 129.58, 127.93, 123.65, 120.19, 82.33, 25.29;ESI-MS m/z (%):407.0[M+1]⁺;Anal.Calcd for: C₁₇H₁₆BrN₃O₄: C, 56.44; H, 4.46; N, 11.61; Found C, 56.52; H, 4.55; N, 11.54.

4-Bromobenzylidene-2-(4-bromophenoxy)-2-methylpropanehydrazide (4f)

White crystal; Yield:69 %; m.p: 201-203 °C;IR (KBr) cm^{-1} : 3041(NH), 1721(C=O), 1583(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 11.66 (s, 1H, NH), 8.37 (s, 1H, CH), 7.85 – 7.58 (m, 2H, ArH), 7.75 – 7.68 (m, 2H, ArH), 7.40 – 7.29 (m, 2H, ArH), 6.82 – 6.72 (m, 2H, ArH), 1.53 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO,100 MHz): 168.52, 152.74, 145.86, 138.22, 132.50, 131.97, 129.58, 129.05, 128.73, 120.19, 82.33, 25.29; ESI-MS m/z (%):441.9[M+1]⁺; Anal. Calcd for: C₁₇H₁₆Br₂N₂O₂: C, 51.60; H, 4.09; N, 7.07; Found C, 51.68; H, 4.15; N, 6.97.

2-(4-Bromophenoxy)-(1-(4-chlorophenyl)ethylidene)-2-methylpropanehydrazide (4g)

Off white powder; Yield: 79 %; m.p: 212-213 °C; IR (KBr) cm^{-1} : 3214(NH), 1757(C=O), 1592(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 10.01 (s, 1H, NH), 7.66 – 7.56 (m, 2H, ArH), 7.33 (ddt, $J=10.3, 8.9, 1.7$ Hz, 4H, ArH), 7.00 – 6.91 (m, 2H, ArH), 2.27 (s, 3H,CH₃), 1.51 (s, 6H, C(CH₃)₂). ^{13}C NMR (DMSO100 MHz): 168.52, 152.74, 150.40, 134.75, 134.26, 132.50, 129.74, 129.58, 129.22, 128.21, 127.43, 120.19, 82.33, 25.29; ESI-MS m/z (%):410.1[M+1]⁺; Anal. Calcd for: C₁₈H₁₈BrClN₂O₂: C, 59.18; H, 4.97; N, 7.67; Found C, 59.22; H, 4.02; N, 7.66.

2-(4-Bromophenoxy)-(1-(2-hydroxyphenyl)ethylidene)-2-methylpropanehydrazide (4h)

White powder; Yield: 77%; m.p:188-190 °C; IR (KBr) cm^{-1} : 3233(NH), 1759(C=O), 1598(C=N); ^1H NMR (DMSO. d_6 300 MHz) δ : 12.63 (s, 1H, NH), 9.64 (s, 1H, OH), 7.47-7.44 (dd, $J=7.7,$

1.6 Hz, 1H, ArH), 7.34 – 7.28 (m, 3H, ArH), 7.06 – 7.03 (m, 1H, ArH), 6.98–6.86(m, 3H, ArH) 2.30 (s, 3H, CH₃), 1.62 (s, 6H, C(CH₃)₂). ¹³C NMR (DMSO, 100 MHz): 169.14, 156.83, 156.26, 152.74, 132.50, 131.90, 129.58, 128.88, 120.27, 120.19, 119.01, 115.15, 82.42, 25.29, 14.41; ESI-MS *m/z* (%): 392.1[M+1]⁺; Anal. Calcd for: C₁₈H₁₉BrN₂O₃: C, 62.33; H, 5.52; N, 8.07; Found C, 62.25; H, 5.44; N, 8.19.

2-(4-Bromophenoxy)-(1-(4-methoxyphenyl)ethylidene)-2-methylpropanehydrazide (4i)

Pale yellow powder; Yield: 62%; m.p.: 191–192 °C; IR (KBr) cm⁻¹: 3224(NH), 1715(C=O), 1598(C=N); ¹H NMR (DMSO, d₆ 300 MHz) δ: 9.56 (s, 1H, NH), 7.85 – 7.81 (m, 2H, ArH), 7.31 – 7.26 (m, 2H, ArH), 6.98 – 6.90 (m, 4H, ArH), 3.85 (s, 3H, CH₃), 3.20 (s, 3H, OCH₃), 1.62 (s, 6H, C(CH₃)₂). ¹³C NMR (DMSO, 100 MHz): 169.14, 161.36, 152.74, 148.77, 132.50, 129.58, 129.47, 128.98, 120.19, 112.93, 82.42, 55.32, 25.29, 13.89; ESI-MS *m/z* (%): 406.1[M+1]⁺; Anal. Calcd for: C₁₉H₂₁BrN₂O₃: C, 58.12; H, 4.59; N, 7.97; Found C, 58.22; H, 4.69; N, 8.07.

2-(4-Bromophenoxy)-2-methyl-(1-phenylethylidene)propanehydrazide (4j)

White crystalline powder; Yield: 81%; m.p.: 202–204 °C; IR (KBr) cm⁻¹: 3321(NH), 1759(C=O), 1591(C=N); ¹H NMR (DMSO, d₆ 300 MHz) δ: 9.63 (s, 1H, NH), 7.87–7.83 (m, 2H, ArH), 7.42 – 7.39 (m, 3H, ArH), 7.31–7.26(m, 2H, ArH) 6.98 – 6.93 (m, 2H, ArH), 2.23 (s, 3H, CH₃), 1.62 (s, 6H, C(CH₃)₂). ¹³C NMR (DMSO, 100 MHz): 169.14, 152.74, 148.92, 136.63, 132.50, 129.90, 129.58, 127.51, 127.28, 120.19, 82.42, 25.29, 13.89; ESI-MS *m/z*: 375.1[M+1]⁺; Anal. Calcd for: C₁₈H₁₉BrN₂O₂: C, 63.24; H, 5.89; N, 7.76; Found C, 63.12; H, 5.75; N, 7.88.

All the synthesized compounds were subjected to biofilm inhibition, antioxidant and mutagenic assay using well-defined literature methods.

Biofilm inhibition activity

A microtitre-plate method was used to determine the inhibitory activity of compounds in biofilm formation. All the compounds showed sensitivity against gram-positive and gram-negative strains of bacteria. Rifampicin (Oxoid, UK) were used as standard antibiotic (positive control) to compare sensitivity of azomethin compounds in biofilm inhibition. It was found that the inhibition assay depends upon the ability of azomethine compounds to form a chelate with bacterial strains and inhibit their growth [22]. Table 2 shows that all compounds are more active

against *E. coli* gram-negative bacterial strain than the gram-positive bacterial strain.

Table 2: Biofilm inhibition percent of benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazides

Compound	Biofilm inhibition % (<i>S.aureus</i>) Gram +ive strain	Biofilm inhibition % (<i>E.coli</i>) Gram -ive strain
4a	57.84	75.33
4b	77.28	79.22
4c	57.39	75.92
4d	57.29	49.46
4e	55.65	74.14
4f	43.18	75.63
4g	63.31	57.07
4h	57.01	60.41
4i	75.51	77.05
4j	61.26	65.22
Positive control (Rifampicin)	84.50	89.01

Anti-oxidant activity

The anti-oxidant activity was evaluated by the radical scavenging ability of compounds by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). In this series, the scavenging potential lies between moderate to good yield as shown in Table 3 when compare with the scavenging potential of standard compound. In Compound **4b** and **4i** the presence of methoxy group at the ortho and para position of the aromatic ring enhance the scavenging ability.

Table 3: Anti-oxidant activity of benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazide

Compound	DPPH (%)
4a	51.22
4b	89.45
4c	23.96
4d	56.56
4e	55.28
4f	62.80
4g	51.00
4h	71.77
4i	82.54
4j	67.12
Ascorbic acid	92.47

Mutagenic activity

Mutagenic screening of compounds was evaluated by Ames bacterial reverse-mutation method. All the compounds were sensitive against both strains of *salmonella* TA98 and TA 100. The result (Table.4) shows that most of the compounds were non-mutagenic or mutagenic for only one strain under assay condition. The

Table 4: Mutagenic screening of benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazide

Variable	TA 98		TA100	
	No. of +ve wells/total no. of wells	Result	No. of +ve wells/total no. of wells	Result
Standard	84/96	Mutagenic	79/96	Mutagenic
Background	11/96	Non-mutagenic	10/96	Non-mutagenic
4a	10/96	Non-mutagenic	26/96	Mutagenic
4b	9/96	Non-mutagenic	10/96	Non-mutagenic
4c	10/96	Non-mutagenic	16/96	Non-mutagenic
4d	22/96	Mutagenic	24/96	Mutagenic
4e	22/96	Mutagenic	10/96	Non-mutagenic
4f	8/96	Non-mutagenic	8/96	Non-mutagenic
4g	5/96	Non-mutagenic	20/96	Mutagenic
4h	10/96	Non-mutagenic	8/96	Non-mutagenic
4i	11/96	Non-mutagenic	9/96	Non-mutagenic
4j	7/96	Non-mutagenic	10/96	Non-mutagenic

compound **4d** only shows greater interaction for DNA to make it stable and causes genetic mutation [23].

DISCUSSION

It was found that organic ligands having azomethine linkage were synthesized by the condensation reaction of amines (primary or secondary) with benzaldehyde/acetophenones. They are diverse class of compounds which have broad range of biological activities such as antibacterial, antifungal, enzyme inhibition, antitumor and antipyretic activities [24]. All the compounds were characterized by spectroscopic techniques. For functional group confirmation, in FTIR spectra the characteristic peak of -NH around 3240 cm^{-1} and azomethine appears at range of $1530 - 1600\text{ cm}^{-1}$ confirmed the synthesis of product, similarly in $^1\text{H NMR}$ the peak of NH_2 around $4.50 - 5.20\text{ ppm}$ was absent, the two prominent singlet peaks of -CH at range of $8.12 - 8.72\text{ ppm}$ and six protons of $\text{-(CH}_3)_2$ support the presence of fibrate moiety and azomethine linkage in benzylidine compound.

The results of biofilm inhibition revealed that all the ligands were active against gram-negative bacterial strain as compare to gram-positive strain. Literature shows that bacterial cell wall is composed of peptidoglycan which is thicker in Gram-positive strains and thinner in Gram-negative strains [25] due to which ligands can easily penetrate through the cell wall of gram-negative bacteria as compare to gram-positive bacteria and disturb cell growth to biofilm formation. Similarly ligands face a barrier for diffusion in Gram positive strain of bacteria [26] and was responsible for the low activity shown by some of the compounds. The compound **4b** and **4i** behave more potent as compare to all other

compounds, it may be due to the presence of oxidative methoxy group whose strong oxidative nature crucially attacked the cell wall of microbes and damaged its growth. [27]

The azomethine compounds have an efficient conjugated system of delocalized electrons and form a stable radicals [28] which makes them an excellent antioxidant. The results in Table 3 shows that the compounds substituted with electron donating group as in **4b** and **4i** have more scavenging activity, while the presence of electron withdrawing substituents (Cl, Br) have the least effect on delocalization and hence, they shows poor activity.

In mutagenic screening result, it was found that most of the compounds shows different mutagenicity behavior against both strains of *Salmonella* TA98 and TA 100.

Literature shows that the position and nature of substituents on phenyl ring greatly effect the mutagenic results [29] as in compound **4d** chloro group is present at Meta position of phenyl ring and causes mutagenicity against both strains of *Salmonella* TA98 and TA 100 while in compound **4e** nitro group is also present at meta position but it is mutagenic only for one strain. It shows that chloro and nitro groups at meta position creates greater interaction with DNA and becomes stable [30] and this stability causes genetic mutation in bacterial strains.

CONCLUSION

Azomethine derivatives of benzylidene-2-(4-bromophenoxy)-2-methyl propane hydrazide have been successfully synthesized by following well-defined procedures and their structures confirmed by IR, NMR and mass spectrometry.

These compounds exhibit significant biological activities which are linked to the position and type of substituents on the structure of azomethine compounds. These findings may be helpful in the synthesis of more potent bioactive azomethine compounds.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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