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Antibacterial and Enzyme Inhibition Studies of *N′*-Substituted Benzylidene-2-(2, 4-Dimethylphenoxy) Acetatohydrazides

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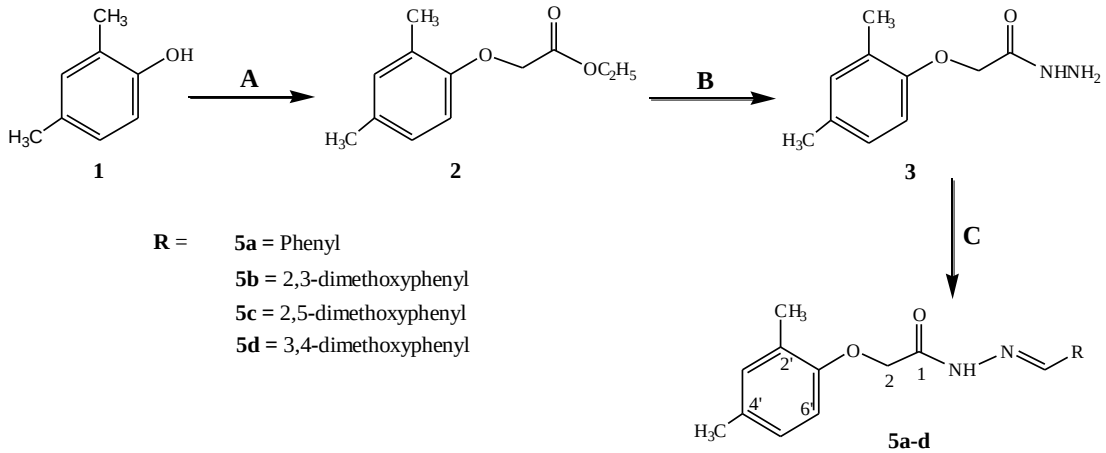
ABSTRACT
The molecules bearing azomethine group are known to possess biological activities. In the present work, the synthesis of *N′*-Substitutedbenzylidene-2-(2, 4-dimethylphenoxy) acetatohydrazide (**5a-d**) has been elaborated using 2,4-Dimethylphenol (**1**) as precursor. The molecule, **1**, was converted to ethyl 2-(2,4-dimethylphenoxy)acetate (**2**) on refluxing with ethyl 2-bromoacetate in ethanol in the presence of KOH. Ethyl ester, **2**, was refluxed with hydrated hydrazine (80%) in ethanol to yield 2-(2,4-dimethylphenoxy) acetohydrazide (**3**). The target molecules, **5a-d**, were synthesized by stirring **3** with phenyl/aryl carboxaldehyde (**4a-d**) in methanol in the presence of glacial acetic acid. The synthesized molecules were characterized by spectral data and evaluated for antibacterial and anti-enzymatic activities.

Keywords: 2, 4-Dimethylphenol, antibacterial activity, anti-enzymatic activity, azomethine, hydrazones.

1. INTRODUCTION
Hydrazone Schiff bases are the molecules with azomethine group and are known to exhibit a broad spectrum of pharmacologically important activities such as antimicrobial, anticancer, anti-inflammatory, antidepressant, anticonvulsant, antioxidant activities and many others. A number of such molecules are playing a key role in medicinal and agricultural chemistry[1-4].

The structural variation has much influence on the biological activities depicted by the synthesized molecules[5-9]. This fact prompted us to synthesize such a series of molecules bearing azomethine and ether linkages for the evaluation of their antibacterial and anti-enzymatic activities. The MIC values of antibacterial activity and IC_{50} values of anti-enzymatic activity rendered these molecules moderately good except one. These molecules can be further activated by synthesizing heterocyclic moieties like 1, 3, 4-Oxadiazole[10-12].

2. RESULTS AND DISCUSSION
A new series of *N′*-Substitutedbenzylidene-2-(2,4-dimethylphenoxy)acetatohydrazide (**5a-d**) was synthesized by outlined Scheme 1. The general procedures and spectral data are expressed in the experimental section.



Scheme-1: Outline for synthesis of *N′*-Substitutedbenzylidene-2-(2,4-dimethylphenoxy)acetatohydrazide (**5a-d**); **Reagents and conditions**: (**A**) Ethyl 2-bromoacetate/EtOH/KOH/Reflux for 6 hours (**B**) 80% Hydrated hydrazine/EtOH/Reflux for 4 hours (**C**) Phenyl/aryl carboxaldehydes (**4a-d**)/MeOH/Glacial acetic acid/Stir for 3-4 hours.

2.1 *Chemistry*
The series of such molecules bearing azomethine and ether linkages was synthesized to evaluate the antibacterial and anti-enzymatic behavior of these molecules. First 2, 4-dimethylphenol (**1**) was converted to ethyl 2-(2,4-dimethylphenoxy)acetate (**2**) on refluxing with ethyl 2-bromoacetate in ethanol in the presence of KOH. The product was separated by filtration after addition of excess of cold distilled water. Second, **2** was refluxed with hydrated

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hydrazine (80%) in ethanol and 2-(2,4-dimethylphenoxy) acetohydrazide (**3**) was afforded through filtration after addition of excess ice cold distilled water. The target molecules, *N′*-Substitutedbenzylidene-2-(2,4-dimethylphenoxy)acetohydrazide (**5a-d**), were yielded by stirring **3** with phenyl/aryl carboxaldehydes (**4a-d**) in methanol in the presence of glacial acetic acid as catalyst at room temperature. The precipitates of products were collected after addition of ice cold distilled water by filtration and dried. Spectroscopic data was used to analyze the structural formulae of synthesized molecules.

The compound, **5a**, white amorphous solid, showed melting point of 120-122 ^{o}C. Its molecular formula, $C_{17}H_{18}N_2O_2$, was established by EI-MS and ^{1}H-NMR spectral data. The stretching frequencies of C=O of carbohydrazide, C=N of azomethine and C-O of ether linkage were observed at 1671, 1682 and 1089 respectively. In EI-MS, molecular ion peak was observed at *m/z* 282 along with two prominent peaks at *m/z* 163 for 2-(2,4-dimethylphenoxy)aceto cation and at *m/z* 119 for benzaldehydehydrazonic cation. The mass fragmentation pattern of **5a** was sketched in Fig. 1. The ^{1}H-NMR spectrum demonstrated three signals for unsubstituted benzylidene ring at δ 8.18 (s, 1H, H-7"), 7.75 (dd, *J* = 7.2, 3.6 Hz, 2H, H-2", H-6") and 7.40-7.38 (m, 3H, H-3" to H-5"); and three signals for substituted phenoxy group at δ 7.00 (s, 1H, H-3'), 6.94 (d, *J* = 7.6 Hz, 1H, H-5') and 6.71 (d, *J* = 8.0 Hz, 1H, H-6') in the aromatic region. The aliphatic region presented three signals for one methylene group of acetamide at δ 4.63 (s, 2H, H-2) and two methyl groups of substituted phenoxy ring at δ 2.29 (s, 3H, CH_3-4') and 2.26 (s, 3H, CH_3-2'). In broad band (BB) and distorsionless enhancement by polarization transfer (DEPT) ^{13}C-NMR spectra, fifteen signals resonated for five quaternary carbons, nine methine carbons, one methylene carbon and two methyl carbons. The five quaternary carbons resonated at δ 164.7 (C-1), 149.4 (C-1'), 133.1 (C-1"), 132.0 (C-4') and 128.8 (C-2'); nine methine carbons resonated seven signals at δ 144.2 (C-7"), 131.8 (C-4"), 130.8 (C-2", C-6"), 127.8 (C-3'), 127.3 (C-5'), 126.8 (C-3", C-5"), 111.9 (C-6'); one methylene carbon appeared at δ 67.8 (C-2); and two methyl carbons appeared at δ 20.4 (C-8') and 16.3 (C-7'). All the spectral data values confirmed the proposed structural formulae of **5a** as *N′*-Benzylidene-2-(2,4-dimethylphenoxy)acetatohydrazide. The structural formulae of all other synthesized compounds were also corroborated through spectral data values, elaborated in experimental section.

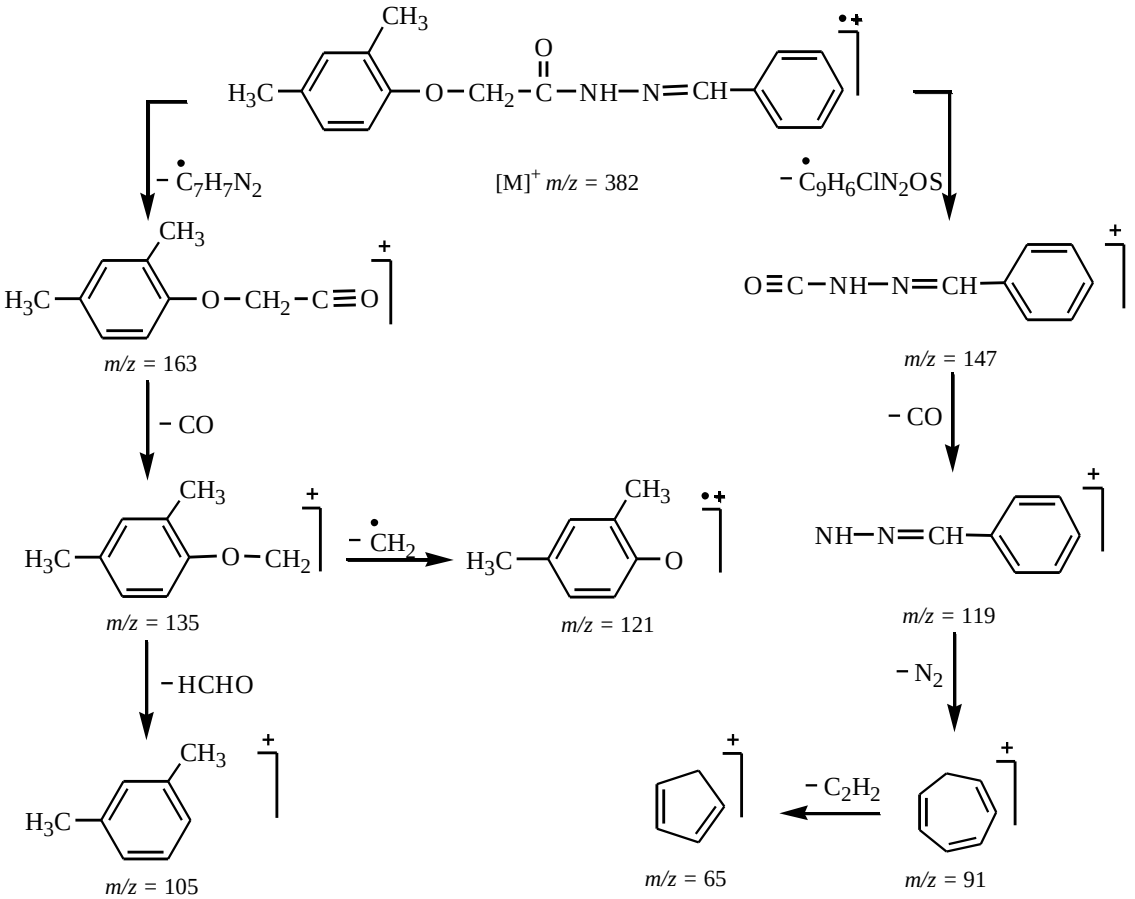

Fig-1: Mass fragmentation pattern of synthesized molecule, **5a**

2.2 *Antibacterial and anti-enzymatic activity (in vitro)*

The results of antibacterial activity are presented as %age inhibition and MIC values (Table 1, 2) relative to the reference standard, ciprofloxacin, and that of lipoxygenase (LOX) inhibition activity are expressed as IC_{50} values relative to baicalein (Table 3). The compounds remained moderately good inhibitors of gram-positive and gram-negative bacterial strains along with better inhibition potential against LOX enzyme.

Table-1: %age inhibition of antibacterial activity

Compound	% Inhibition				
	S. typhi (-)	***E. coli (-)***	***B. subtilis (+)***	***S. aureus (+)***	***P. aeruginosa (-)***
2	72.22±1.78	71.34±0.54	68.05±1.45	37.89±4.21	73.45±1.29
3	58.11±1.11	64.90±3.42	65.30±2.50	48.47±3.21	71.70±2.53
5a	39.11±3.56	13.22±4.31	31.05±3.95	40.37±4.89	29.23±2.25
5b	67.33±0.78	73.02±1.24	63.30±1.90	60.47±1.95	64.48±0.36
5c	65.56±1.89	68.12±0.59	62.15±0.55	60.95±2.53	62.01±1.39
5d	45.22±0.54	57.28±2.43	49.80±1.80	30.05±5.00	49.23±1.08
Ciprofloxacin	**92.43±1.07**	**90.67±0.65**	**91.99±2.00**	**91.00±1.65**	**90.33±0.22**

The MIC values explained the inhibition potential of the compounds. The molecules, **2** and **3** showed better potential against *E. coli* and *P. aeruginosa* with MIC values of 11.18±3.25 µM and 11.30±0.58 µM relative to 7.96±1.14 µM and 8.05±1.60 µM but moderate against *S. typhi* and *B. subtilis*. Both molecules executed no activity at all against *S. aureus*. The molecules, **5b** and **5c** showed good activity against all the bacterial strains taken into account and also comparable to that of the reference standard. These two molecules owe their activity because of substitution at 2nd position and/or 3rd or 5th of benzylidene group which is absent in remaining ones. The substitution at 4th position and/or 2nd or 3rd position rendered the molecules less reactive. The compounds, **5a** and **5d** remained the least active as first one showed no activity at all and the second one very moderate activity against *E. coli* only with MIC value of 13.16±1.68 µM relative to 7.96±1.14 µM of ciprofloxacin.

Table-2: MIC values of antibacterial activity

Compound	MIC				
	S. typhi (-)	***E. coli (-)***	***B. subtilis (+)***	***S. aureus (+)***	***P. aeruginosa (-)***
2	13.94±1.00	9.95±2.50	16.25±2.25	-	10.53±4.08
3	12.28±5.00	11.18±3.25	15.17±5.00	-	11.30±0.58
5a	-	-	-	-	-
5b	11.39±2.30	10.72±1.31	11.84±2.46	13.23±2.20	12.13±1.08
5c	10.60±5.00	10.52±2.38	12.31±4.24	11.54±1.79	11.27±3.08
5d	-	13.16±1.68	-	-	-
Ciprofloxacin	**8.00±2.54**	**7.96±1.14**	**8.32±1.00**	**7.43±0.45**	**8.05±1.60**

The enzyme inhibition potential has been evaluated against lipoxygenase (LOX) enzyme. The molecules, **5a**, **5b** and **5c** were found to be better inhibitor of this enzyme but other three molecules showed the least activity. Among the whole series, the best inhibitor was **5c** showing IC_{50} value of 132.5±0.52 µM relative to baicalein with IC_{50} value of 22.4±1.3 µM. The reactivity order of all the molecules was **5c>5a>5b>5d=3>2**.

Table-3: Enzyme inhibition against LOX

Compound	LOX		
	Conc. (mM)	**Inhibition (%)**	**IC_{50} (µM)**
2	0.5	27.44±0.34	--
3	0.5	43.56±0.78	>500
5a	0.5	98.44±0.12	211.3±0.82
5b	0.5	72.18±0.26	312.3±0.78
5c	0.5	93.44±0.65	132.5±0.52
5d	0.25	52.66±0.23	>500
Baicalein	**0.5**	**93.76±1.21**	**22.4±1.3**

Note: LOX = Lipoxygenase. IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

3. CONCLUSION

All the compounds were synthesized with better yields and characterized by structural formulae through spectral data values. Because of azomethine group, the activity was supposed to be much better and the results showed the expected values up to much extent for antibacterial and anti-enzymatic potentials. The substitution at 2nd and other positions except 4th of benzylidene group rendered the molecules active against bacterial strains and lipoxygenase enzyme. These can be further evaluated for *in vivo* activity and hence these might be capable for the drug discovery pathway regarding pharmacological research.

4. MATERIALS AND METHODS

4.1 *General*

The IR spectral data was collected by KBr pellet method on Jasco-320-A spectrophotometer. NMR spectral data was collected for PNMR and CNMR in $CHCl_3$-d_1 on Bruker spectrometer at 400 and 100 MHz respectively, with δ-values

in ppm and *J*-values in Hz. EIMS spectral data were collected on JMS-HX-110 spectrometer. Melting points of all the synthesized compounds were noted on Griffin-George apparatus. TLC plates with silica gel 60 F_{254} were used and visualized under UV at 254nm with solvent system of ethyl acetate and *n*-hexane. The chemical reagents were Alfa Aesar and Sigma-aldrich branded bought from local suppliers and analytical grade solvents were employed.

4.2 *Procedure for synthesis of ethyl 2-(2,4-dimethylphenoxy)acetate (2)*

2,4-Dimethylphenol(**1**; 2.5 mmol) was dissolved in 30 mL ethanol in a 100 mL round bottom (RB) flask. Solid KOH (2.5 mmol) was added and system was refluxed for 0.5 hour. Then electrophile, ethyl 2-bromoacetate (2.5 mmol) was added and system was further refluxed for 6 hours. The reaction was supervised by TLC. After single spot on TLC, system was left to cool down to room temperature. Reaction mixture was transferred to a separating funnel (250 mL) followed by addition of ice cold distilled water (50 mL) and chloroform (30 mL). The system was shaken vigorously and left till appearance of two layers. The chloroform layer was separated and the title compound was afforded after evaporation. Light brown liquid; Yield: 86%; Molecular formula: $C_{12}H_{16}O_3$; Molecular weight: 208 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3057 (Ar C-H), 1733 (ester C=O), 1583 (Ar C=C), 1121 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 6.95 (s, 1H, H-3'), 6.85 (d, *J* = 8.0 Hz, 1H, H-5'), 6.60 (d, *J* = 8.0 Hz, 1H, H-6'), 4.58 (s, 2H, H-2), 4.24 (q, *J* = 7.2 Hz, 2H, H-1''), 2.23 (s, 3H, CH_3-4'), 2.20 (s, 3H, CH_3-2'), 1.28 (t, *J* = 7.2 Hz, 3H, CH_3-2''); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ_C/ppm): 169.5 (C-1), 149.7 (C-1'), 132.3 (C-4'), 128.5 (C-2'), 127.4 (C-3'), 126.5 (C-5'), 112.1 (C-6'), 67.9 (C-2), 63.3 (C-1''), 20.3 (C-8'), 16.2 (C-7'), 14.5 (C-2''); EIMS (*m/z*): 208 $[M]^+$, 163 $[C_{10}H_{11}O_2]^+$, 135 $[C_9H_{11}O]^+$, 121 $[C_8H_9O]^+$, 105 $[C_8H_9]^+$, 93 $[C_7H_9]^+$, 29 $[C_2H_5]^+$.

4.3 *Procedure for synthesis of 2-(2,4-dimethylphenoxy)acetohydrazide (3)*

Ethyl ester, **2** (2.1 mmol) was mixed with 20 mL ethanol in a 100 mL RB flask followed by the addition of 80% hydrated hydrazine (2.1 mmol). The reaction mixture was refluxed for 4 hours and monitored by TLC. After confirmation by TLC, excess of cold distilled water was poured to the reaction mixture along with shaking. The settled precipitates of title compound were collected by filtration and washed by *n*-hexane. Pink amorphous solid; Yield: 80%; Melting point: 134-136 ^{o}C; Molecular formula: $C_{10}H_{14}N_2O_2$; Molecular weight: 194 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3412 (N-H), 3071 (Ar C-H), 1667 (amide C=O), 1595 (Ar C=C), 1155 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 6.96 (s, 1H, H-3'), 6.93 (d, *J* = 8.4 Hz, 1H, H-5'), 6.63 (d, *J* = 8.4 Hz, 1H, H-6'), 4.52 (s, 2H, H-2), 2.24 (s, 3H, CH_3-4'), 2.21 (s, 3H, CH_3-2'); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ_C/ppm): 164.8 (C-1), 148.6 (C-1'), 132.7 (C-4'), 128.3 (C-2'), 127.1 (C-3'), 126.2 (C-5'), 112.0 (C-6'), 67.7 (C-2), 20.1 (C-8'), 16.2 (C-7'); EIMS (*m/z*): 194 $[M]^+$, 192 $[C_{10}H_{12}N_2O_2]^+$, 163 $[C_{10}H_{11}O_2]^+$, 135 $[C_9H_{11}O]^+$, 121 $[C_8H_9O]^+$, 105 $[C_8H_9]^+$, 93 $[C_7H_9]^+$.

4.4 *General procedure for synthesis of N'-Substitutedbenzylidene-2-(2,4-dimethyl phenoxy)acetatohydrazide (5a-d)*

Compound **3** (0.4 mmol) was taken in 50 mL RB flask and mixed with 15 mL methanol. Two drops of glacial acetic acid were added to catalyze the reaction. The phenyl/aryl carboxaldehyde (**4a-d**, 0.4 mmol) were added and the reaction mixture was stirred for 3-4 hours. After complete reaction, supervised by TLC, excess of cold distilled water was added to the reaction mixture along with shaking till precipitation. The precipitates of the synthesized compounds were filtered, washed and dried for spectral and biological analysis.

4.4.1 *N'-Benzylidene-2-(2,4-dimethylphenoxy)acetatohydrazide (5a)*

White amorphous solid; Yield: 78%; Melting point: 120-122 ^{o}C; Molecular formula: $C_{17}H_{18}N_2O_2$; Molecular weight: 282 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3386 (N-H), 3111 (Ar C-H), 1671 (amide C=O), 1682 (C=N), 1581 (Ar C=C), 1089 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 8.18 (s, 1H, H-7''), 7.75 (dd, *J* = 7.2, 3.6 Hz, 2H, H-2'', H-6''), 7.40-7.38 (m, 3H, H-3'' to H-5''), 7.00 (s, 1H, H-3'), 6.94 (d, *J* = 7.6 Hz, 1H, H-5'), 6.71 (d, *J* = 8.0 Hz, 1H, H-6'), 4.63 (s, 2H, H-2), 2.29 (s, 3H, CH_3-4'), 2.26 (s, 3H, CH_3-2'); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ_C/ppm): 164.7 (C-1), 149.4 (C-1'), 144.2 (C-7''), 133.1 (C-1''), 132.0 (C-4'), 131.8 (C-4''), 130.8 (C-2'', C-6''), 128.8 (C-2'), 127.8 (C-3'), 127.3 (C-5'), 126.8 (C-3'', C-5''), 111.9 (C-6'), 67.8 (C-2), 20.4 (C-8'), 16.3 (C-7'); EIMS (*m/z*): 282 $[M]^+$, 163 $[C_{10}H_{11}O_2]^+$, 135 $[C_9H_{11}O]^+$, 121 $[C_8H_9O]^+$, 119 $[C_7H_7N_2]^+$, 105 $[C_8H_9]^+$, 104 $[C_7H_6N]^+$, 93 $[C_7H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

4.4.2 *N'-(2,3-Dimethoxybenzylidene)-2-(2,4-dimethylphenoxy)acetatohydrazide (5b)*

Cream white amorphous solid; Yield: 82%; Melting point: 150-152 ^{o}C; Molecular formula: $C_{19}H_{22}N_2O_4$; Molecular weight: 342 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3379 (N-H), 3167 (Ar C-H), 1650 (amide C=O), 1676 (C=N), 1592 (Ar C=C), 1104 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ_H/ppm): 8.43 (s, 1H, H-7''), 7.68 (dd, *J* = 8.8, 0.8 Hz, 1H, H-6''), 7.41 (dd, *J* = 8.4, 1.6 Hz, 1H, H-4''), 7.06 (t, *J* = 8.0 Hz, 1H, H-5''), 7.00 (s, 1H, H-3'), 6.94 (d, *J* = 7.6 Hz, 1H, H-5'), 6.72 (d, *J* = 8.0 Hz, 1H, H-6'), 4.63 (s, 2H, H-2), 3.87 (s, 3H, CH_3-9''), 3.85 (s, 3H, CH_3-8''), 2.30 (s, 3H, CH_3-8'), 2.26 (s, 3H, CH_3-7'); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ_C/ppm): 164.6 (C-1), 152.6 (C-1'), 144.9 (C-7''), 140.0 (C-3''), 138.2 (C-2''), 132.0 (C-3'), 131.8 (C-4'), 131.7 (C-2'), 127.5 (C-5'), 126.4 (C-1''), 124.2 (C-6''), 118.7 (C-4''), 114.4 (C-5''), 112.1 (C-6'), 68.0 (C-2), 61.7 (C-8''), 55.8 (C-9''), 20.4 (C-8'), 16.3 (C-7'); EIMS (*m/z*): 342 $[M]^+$, 179 $[C_9H_{11}N_2O_2]^+$,

164 $[C_9H_{10}NO_2]^+$, 163 $[C_{10}H_{11}O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 137 $[C_8H_9O_2]^+$, 135 $[C_9H_{11}O]^+$, 121 $[C_8H_9O]^+$, 105 $[C_8H_9]^+$, 93 $[C_7H_9]^+$.

4.4.3 *N'-(2, 5-Dimethoxybenzylidene)-2-(2,4-dimethylphenoxy)acetatohydrazide (5c)*

Yellowish white amorphous solid; Yield: 89%; Melting point: 156-158 ^oC; Molecular formula: $C_{19}H_{22}N_2O_4$; Molecular weight: 342 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3397 (N-H), 3176 (Ar C-H), 1666 (amide C=O), 1678 (C=N), 1562 (Ar C=C), 1159 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 8.47 (s, 1H, H-7"), 7.58 (d, J = 3.2 Hz, 1H, H-6"), 7.00 (s, 1H, H-3'), 6.95-6.92 (m, 2H, H-4", H-5'), 6.83 (d, J = 9.2 Hz, 1H, H-3"), 6.72 (d, J = 8.4 Hz, 1H, H-6'), 4.62 (s, 2H, H-2), 3.81 (s, 3H, CH_3-9"), 3.80 (s, 3H, CH_3-8"), 2.29 (s, 3H, CH_3-8'), 2.26 (s, 3H, CH_3-7'); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ_C/ppm): 164.4 (C-1), 152.8 (C-1'), 147.3 (C-5"), 144.5 (C-7"), 138.4 (C-2"), 131.5 (C-4'), 130.8 (C-2'), 129.2 (C-3'), 127.5 (C-5'), 126.3 (C-1"), 117.6 (C-4"), 112.7 (C-6'), 112.3 (C-6"), 112.0 (C-3"), 68.3 (C-2), 55.9 (C-9"), 61.5 (C-8"), 20.3 (C-8'), 16.4 (C-7'); EIMS (*m/z*): 342 $[M]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 164 $[C_9H_{10}NO_2]^+$, 163 $[C_{10}H_{11}O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 137 $[C_8H_9O_2]^+$, 135 $[C_9H_{11}O]^+$, 121 $[C_8H_9O]^+$, 105 $[C_8H_9]^+$, 93 $[C_7H_9]^+$.

4.4.4 *N'-(3, 4-Dimethoxybenzylidene)-2-(2,4-dimethylphenoxy) acetatohydrazide (5d)*

Brownish yellow amorphous solid; Yield: 76%; Melting point: 148-150 ^oC; Molecular formula: $C_{19}H_{22}N_2O_4$; Molecular weight: 342 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3357 (N-H), 3143 (Ar C-H), 1659 (amide C=O), 1677 (C=N), 1590 (Ar C=C), 1217 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 8.09 (s, 1H, H-7"), 7.46 (d, J = 1.6 Hz, 1H, H-1"), 7.12 (dd, J = 8.4, 2.0 Hz, 1H, H-6"), 7.00 (s, 1H, H-3'), 6.95 (d, J = 8.4 Hz, 1H, H-5'), 6.85 (d, J = 8.4 Hz, 1H, H-4"), 6.71 (d, J = 8.0 Hz, 1H, H-6'), 4.62 (s, 2H, H-2), 3.93 (s, 3H, CH_3-8"), 3.90 (s, 3H, CH_3-9"), 2.29 (s, 3H, CH_3-8'), 2.26 (s, 3H, CH_3-7'); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ_C/ppm): 164.1 (C-1), 152.9 (C-1'), 149.7 (C-3"), 146.6 (C-4"), 144.3 (C-7"), 132.4 (C-4'), 129.2 (C-1"), 126.5 (C-2'), 124.2 (C-3'), 122.8 (C-5'), 122.1 (C-6"), 112.4 (C-6'), 108.6 (C-5"), 107.1 (C-2"), 68.6 (C-2), 61.4 (C-8"), 55.7 (C-9"), 20.1 (C-8'), 16.8 (C-7'); EIMS (*m/z*): 342 $[M]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 164 $[C_9H_{10}NO_2]^+$, 163 $[C_{10}H_{11}O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 137 $[C_8H_9O_2]^+$, 135 $[C_9H_{11}O]^+$, 121 $[C_8H_9O]^+$, 105 $[C_8H_9]^+$, 93 $[C_7H_9]^+$.

5. BIOLOGICAL ACTIVITY ASSAYS

5.1 *Antibacterial Activity*

The antibacterial activity was assayed by the reported method[12].

5.2 *Anti-enzymatic Activity*

The LOX enzyme inhibition assay was performed by Baylac and Racine method[13].

5.3 *Statistical Analysis*

Each experiment was executed in triplicate and statistically analyzed by ME 2010. All the results are tabulated as mean ±SEM.

6. ACKNOWLEDGEMENT

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