



# Synthesis, *in silico* study and *in vitro* anti-microbial evaluation of some new N-benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides analogs

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## ABSTRACT

In bacterial resistance duty, the prompt increase against existent anti-microbial drugs is a challenging universal health problem. Bacteria represent a highly significant threat globally to healthcare-associated infections, also responsible for the majority of hospital infections, which leads to an increase in the mortality and burden of worldwide healthcare duty. In this investigation, we reported the synthesis novel substitutes of phenoxy hydrazide analogs (**6a-f**) and have been screened for *in vitro* anti-bacterial and anti-fungal activities to determine the inhibition zone by using the paper disk agar diffusion technique and broth dilution to evaluate the minimum inhibitory concentration values. The structure-activity relationship suggest that among the series (**6a-f**), compounds (**6e**) with two chloro groups and (**6f**) with four fluoro groups showed good inhibition against pathogenic microbes. Furthermore, these results were also confirmed by the *in silico* study. Based on this studies, there are a scopes of developing compounds (**6e**) and (**6f**) into potent anti-microbial drugs in the near future.

## INTRODUCTION

A pathogen infection is a microorganism that is able to cause most of the life-threatening disease and have become a serious public medical issue in the current days (Spellberg *et al.*, 2008). Since there are huge interests in the up growth of the novel anti-microbial drugs future as a result of the gradual growth of bacterial and fungal resistance to a wide range of factors. *Staphylococcus aureus* is the main global, as well as extremely dangerous human pathogens that cause the variety of diseases, tend to range from fairly acute skin infections to deadly pneumonia and is one of the world's most serious causes of morbidity (Al-Ostoot *et al.*, 2018; Arias and Murray, 2009; Perron *et al.*, 2013). In addition, with the worrisome increase in multi-drug

resistant bacteria species, doctors are now ultimately obliged to refer patients to the second or even third antibiotic available option to combat the highly resistant of pathogens (Harrison and Svec, 1998). Hence, it is vitally important to reveal and develop new anti-microbial agents to combat these diseases (Vaikunthanathan *et al.*, 2016). Natural products show to be the best source of new bioactive substrates in the design of anti-microbial pharmaceuticals; furthermore, their synthetic and semi-synthetic derivative products have also a wonderful clinical value as anti-biotic agents (Dhami *et al.*, 2014). The synthesized compounds with hydrazide moiety exhibited diverse pharmacological activities such as anti-oxidants (Bär *et al.*, 2018), analgesic (Sheykhi-Estalkhjani *et al.*, 2018), anti-bacterial (Gholivand *et al.*, 2010; Morjan *et al.*, 2014; Ozdemir *et al.*, 2015), anti-fungal (Backes *et al.*, 2014), anti-HIV (Yang *et al.*, 2016), anti-tuberculosis (Velezheva *et al.*, 2016), anti-genotoxic (Bordoloi *et al.*, 2009), anti-neoplastic drugs agents (Mohammed *et al.*, 2017; 2018). Due to their wide potential uses as a medicinal drug, special attention has also been given to phenoxy acetic acid as well as its derivative products. In recent years, phenoxy hydrazides and

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the corresponding phenoxy acetic acid condensation derivatives draw as much attention throughout the field of synthetic and medicinal chemistry because of their different biological actions. Earlier our group has reported various compounds bearing phenoxy moiety as anti-microbial (Khanum *et al.*, 2003), anti-oxidant (Prashanth *et al.*, 2013), anti-inflammatory (Khanum *et al.*, 2008) and anti-cancer agents (Kim *et al.*, 2016; Puttaswamy *et al.*, 2018; Zabiulla *et al.*, 2016). As our ongoing work for a search of new drug (Kumar *et al.*, 2018a; 2018b; Kumara *et al.*, 2019), we have synthesis initially active phenoxy acetic hydrazide moiety (4) and then coupled with different substituted aromatic acids having an active group members, such as fluoro, chloro, bromo, and nitro to produce the phenoxy hydrazide analogs (6a–f, Fig. 1) and evaluated their anti-microbial activity.

## RESULTS AND DISCUSSION

### Chemistry

Scheme 1 outlined the synthesis reactions of the title compounds N-benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (6a–f). The compounds (6a–f) were obtained by refluxing of 4-chloro phenol (1) with ethyl chloroacetate (2) in the presence of anhy.  $K_2CO_3$  to give the desired ethyl-2-(4-chlorophenoxy) acetate (3) which to treat by hydrazine hydrate in the presence of ethanol as a solvent to afford (4-chloro-phenoxy)-acetic acid hydrazide (4). Finally, substituted benzoic acids (5a–f, 2.2 mmol) in the solvent of dichloromethane (DCM) (25 ml) was stirred at 30°C, and then the lutidine (3 mmol) was added to the mixture, which then followed by adding of hydrazide compounds with (4, 2.2 mmol). The mixture of the reaction stirred for 20 minutes at 25°C–30°C, and then, the environmental condition of reaction has been changed to cooled in 0°C–5°C, 2-(1H-benzotriazole-1-yl)-1,1,3,3 tetramethylammonium tetrafluoroborate (TBTU, 2 mmol) was then added over a period of 20 minutes with keeping the temperature condition below than 5°C. The reaction has been fully stirred for 13–15 hours at the same condition. Thin layer chromatography (TLC) technique is often used to monitor the progress of chemical reactions and identify compounds present in a given mixture using mobile phase system of hexane: ethyl acetate (6:3). The remaining solvent was completely eliminated at reduced

pressure by adding mashed ice to the mixture with stirring for a few minutes. The solid was filtered and recrystallized with ethanol to obtain compounds (6a–f) (Geetha *et al.*, 2019). compounds structure of 6a–f were confirmed by using some spectroscopy as nuclear magnetic resonance (NMR), infrared (IR), and liquid chromatography–mass spectrometry (LC-MS) techniques. The compound of ester (3) formation was confirmed by observing the disappearance of compound (1) OH stretching and the appearance of new carbonyl group stretching band in the IR absorption spectrum for the ester group at 1,670  $cm^{-1}$ . Compounds proton for NMR spectrum can be revealed by proton disappeared of broad singlet in the hydroxy group of phenol compound (1) and a triplet  $CH_2$  and quartet  $CH_3$  for and protons at  $\delta$  1.10 and  $\delta$  4.13 ppm, respectively, was appeared in compound (3). In addition, the mass spectrum gave significant stable  $M^+$  and  $M^{+2}$  peaks at  $m/z$  214 and 216, respectively, which can clearly confirmed the ester of compound (3) formation. In the same way, the spectrum of the hydrazide compound (4) confirmed by the presentation of NH and  $NH_2$  stretching bands in the range of 3,100–3,205  $cm^{-1}$  and 3,305–3,415  $cm^{-1}$ , respectively, in the IR spectrum. Furthermore, in the proton NMR of compound (4), the appearance of two singlet peaks  $NH_2$  and NH protons at  $\delta$  4.33 and  $\delta$  9.80 ppm, respectively, was confirmed. Also, the mass spectrum of compound (4) showed significant  $M^+$  and  $M^{+2}$  peaks at  $m/z$  200 and 202, respectively. Furthermore, (6a) compound spectrum was considered as a model to explain the spectral data for the (6a–f) compounds that was easily revealed with respectively disappearance of  $NH_2$  and COOH stretching bands of the compounds (4) and (5a), and the appearance of NH stretching band at the range of 3,210–3,320  $cm^{-1}$  in the IR spectrum. NMR spectrum was confirmed by the disappearance of  $NH_2$  proton of (4) and COOH protons of (5a) and appearance of NH proton peak at  $\delta$  10.71 ppm. Besides, there is an increase in aromatic protons in range of  $\delta$  6.98–8.20 ppm and the mass spectrum also showed significant stable  $M^+$  and  $M^{+2}$  peaks at  $m/z$  384 and 486, respectively, which clearly confirmed the formation of title product (6a).

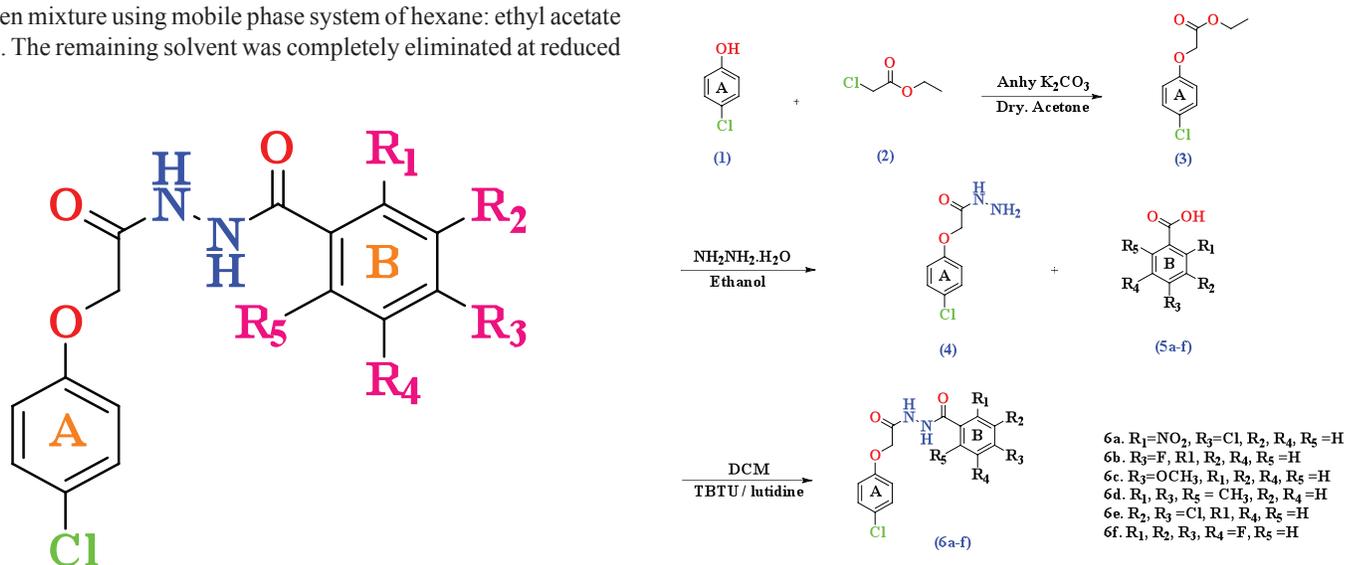


Figure 1. N-Benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides analogs.

Scheme 1. Synthesis of novel N-benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides analogs (6a–f).

## Biology

The title compounds (**6a–f**) were screened for their anti-microbial activity using the cup-plate method in the sabouraud agar medium. From anti-bacterial evaluation results against *B. cereus*, *E. coli*, *S. aureus*, and *P. aeruginosa* it is evident that most of the title compounds has exhibited comparable activity with reference to the standard compound ampicillin. The title compounds were also evaluated for their anti-fungal activity against *A. niger*, *A. flavus*, *R. stolonifer*, *F. oxysporum* using, and fluconazole as standard compound shown in Table 1. The zone of inhibition (mm) of each compound was determined and compared with the standard drugs. Compounds bearing two chloro groups at position 3 and 4 in ring **B** (**6e**) and four fluoro groups at position 2, 3, 4, and 5 in ring **B** (**6f**) were found to be highly active against both bacterial (Fig. 2) and fungal strain (Fig. 3). The results indicated that compounds bearing electron withdrawing groups exhibited good activity against both of the strains. Besides, the other compounds exhibited moderated anti-bacterial and anti-fungal activities. Furthermore, minimum fungicidal concentration (MFC), MIC, and finally minimum bacterial concentration (MBC) assays designed for all the synthesized compounds of (**6a–f**) were also carried out in which compounds (**6e**) and (**6f**) have shown less MIC, MBC, and MFC values compared to the other compounds shown in Tables 2 and 3. Thus, compounds of (**6e**) and (**6f**) can be used in near future for the treatment of pathogenic infection diseases.

## SAR study

Through the review of literature which showed that the phenoxy acetic acid hydrazide and their derivatives has a potential pharmacological activity as anti-microbial, anti-oxidants, and anti-cancer. This study involves the multistep synthesis of phenoxy acetic acid hydrazide hybrids with phenyl acid derivatives Scheme 1. A new hydrazide derivatives *N*-benzoyl-*N'*-[2-(4-chloro-phenoxy)-acetyl]-hydrazides (**6a–f**), were synthesized, characterized and estimated for their anti-

microbial actions against of four bacterial and four fungal strains. Compound (**6e**) bearing two chloro groups at positions 3 and 4 in ring **B** has shown slightly more activity compared to compound (**6f**) against all the four bacterial and fungal strains as shown in Table 1. For that reason, (**6e**) was chosen as a potent compound, based on its significant structure-activity relationship (SAR) and further verified by *in-silico* study docking with 1kznprotein.

## Compound (6e) interacts with 1kzn protein in-silico

By using the modeling study, compound (**6e**) was docked into the active site of the protein 1kzn subunit and then was analyzed for binding free energy and their interactions with the receptor. The ligand (**6e**) interacts through hydrogen bonding with the DNA-gyrase binding site. The distance of hydrogen bonds was shorter than 3.5 Å. The best conformations from the docking procedure with the best scored pose and the lowest binding energy (−6.5 kcal/mol). The results confirmed that the *in-silico* study was matched with *in-vitro* study as shown in Figure 4.

## MATERIALS AND METHODS

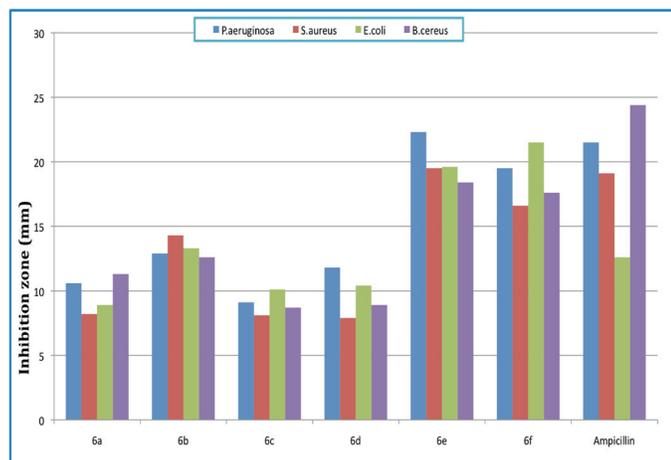
All solvents and reagents, phenols substituted, ethyl chloro acetate, hydrazine hydrate, acids, 2,6 lutidine, and O-(benzotriazol-1-yl)-*N,N,N',N'*-TBTU, were purchased from Sigma Aldrich Chemicals Pvt Ltd. with a purity of 90%–99%. Analytical TLC was performed on 0.30 mm of silica gel plates (Merck 60 F 254 ) by using the solvent of ethyl acetate: hexane (2:7) system and visualized by UV-light. The melting point has been determined using a digital thermometer depending melting point device based on the Chemi Line CL725 microcontroller. NMR spectrum was recorded on a VNMRS-400 MHz Agilent-NMR spectrophotometer in CDCl<sub>3</sub> or dimethyl sulfoxide (DMSO). The potassium bromide pellet method use to collected the IR spectrum on the Cary 630 FTIR Agilent spectrophotometer, VG70-70H spectrometer was used

**Table 1.** Anti-microbial activity of compounds (**6a–f**) (zone of inhibition, mm) against clinical pathogens.

Compounds		Bacterial strains				Fungal strains			
		<i>B. cereus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>R. stolonifer</i>	<i>F. oxysporum</i>
6a	ZI	11.3 ± 0.31	8.9 ± 0.41	8.2 ± 0.51	10.6 ± 0.25	10.3 ± 0.35	11.3 ± 0.41	12.3 ± 0.51	9.2 ± 0.35
	AI	0.463	0.705	0.430	0.493	0.584	0.662	0.802	0.425
6b	ZI	12.6 ± 0.52	13.3 ± 0.52	14.3 ± 0.40	12.9 ± 0.31	18.5 ± 0.54	17.5 ± 0.56	15.2 ± 0.91	16.3 ± 0.51
	AI	0.516	1.053	0.750	0.599	1.049	1.025	0.992	0.753
6c	ZI	8.7 ± 0.40	10.1 ± 0.34	8.1 ± 0.50	9.1 ± 0.34	15.6 ± 0.10	12.1 ± 0.48	12.2 ± 0.49	14.6 ± 0.81
	AI	0.356	0.799	0.424	0.422	0.724	0.708	0.795	0.674
6d	ZI	8.9 ± 0.31	10.4 ± 0.41	7.9 ± 0.49	11.8 ± 0.44	9.7 ± 0.31	11.7 ± 0.42	12.1 ± 0.46	8.3 ± 0.38
	AI	0.364	0.823	0.414	0.548	0.450	0.685	0.789	0.383
6e	ZI	18.4 ± 0.56	19.6 ± 0.65	19.5 ± 0.52	22.3 ± 0.42	19.7 ± 0.11	21.5 ± 0.32	20.1 ± 0.62	20.9 ± 0.11
	AI	0.549	1.552	1.023	1.036	1.117	1.260	1.311	0.873
6f	ZI	17.6 ± 0.43	21.5 ± 0.86	16.6 ± 0.13	19.5 ± 0.52	20.1 ± 0.62	18.3 ± 0.54	18.8 ± 0.52	20.4 ± 0.86
	AI	0.721	1.702	0.870	0.906	1.140	1.072	1.226	1.035
Ampicillin		24.4 ± 0.53	12.63 ± 0.64	19.07 ± 0.33	21.52 ± 0.43	-	-	-	-
Fluconazole		-	-	-	-	21.52 ± 0.63	17.07 ± 0.52	15.33 ± 0.84	21.94 ± 0.30

Where AI = activity index and ZI = zone of inhibition.

Standards: Fluconazole (1.0 mg/disc), Ampicillin (1.0 mg/disc); AI- activity index = ZI of test sample/ZI of standard. Values are mean of triplicate of the readings (mean ± SD).



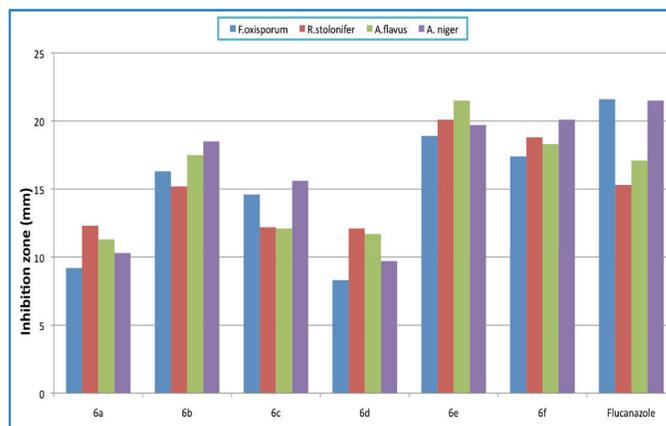
**Figure 2.** Represents that comparison among six compounds of N-benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides (**6a-f**) analogs against bacterial strains.

to obtained Mass spectrum data. Elemental analysis results are within 0.5% of the calculated value.

## Chemistry

### General synthetic procedure for ethyl 2-(4-chlorophenoxy)acetate (**3**)

A reaction mixture of 4-chlorophenol (**1**, 0.030 mol), ethylchloro acetate (0.032 mol) and potassium carbonate (0.075 mol, anhydrous) was refluxed in the solvent of dry acetone (40 ml) for 8 hours to obtained phenoxy acetic ethyl ester (**3**). TLC technique used to monitor the completion of the reaction by using the solvent system of hexane: ethyl acetate (3:1). The mixture of the reaction was cooled and the solvent was removed by distillation method after completion of the reaction. To remove potassium carbonate, the residual mass was poured into cold water and extracted with ether (3 × 20 ml). The ether layer was washed with 10% of sodium hydroxide solution (3 × 20 ml), followed by water (3 × 20 ml),



**Figure 3.** Represents that comparison among six compounds of N-benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides (**6a-f**) analogs against fungal strains.

then to remove moisture, passed over anhydrous sodium sulfate and evaporated to obtained liquid ester compound (**3**).

### Ethyl 2-(4-chlorophenoxy)acetate (**3**)

Yield 70%; B.P. 177°C; FT-IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 1,732–1,752 (ester, C=O), 1,132 (ester, C-O);  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 1.19 (*t*, 3H, CH<sub>3</sub> of ester), 4.13 (*q*, 2H, CH<sub>2</sub> of ester), 4.76 (*s*, 2H, OCH<sub>2</sub>), 6.67–6.88 (*d*, 2H, *J* = 8 Hz, Ar-H), 7.41–7.44 (*d*, 2H, *J* = 8 Hz, Ar-H); LC-MS *m/z* 214 [ $\text{M}^+$ ], 216 [ $\text{M}^{+2}$ ] (68), 128 (100), 184 (38), 186 (12), 170 (40), 170 (6). Anal.Calcd.for C<sub>10</sub>H<sub>11</sub>ClO<sub>3</sub> (214): C, 55.96; H, 5.17. Found: C, 55.87%; H, 5.13%.

### General synthetic procedure for (4-chloro-phenoxy)-acetic acid hydrazide (**4**)

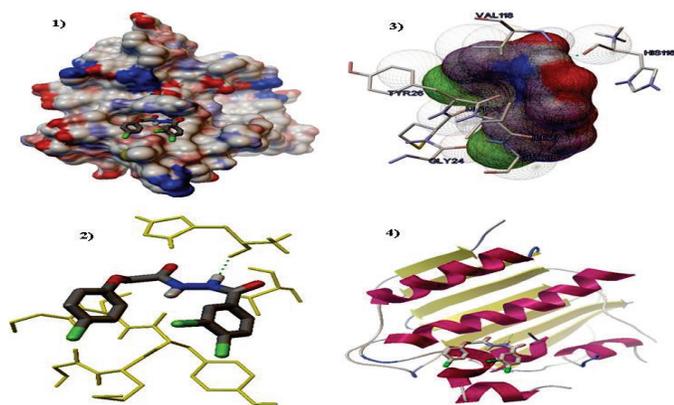
The volume of (0.018 mol) hydrazine hydrate was added to a solution of compound (**3**) (0.018 mol) in absolute ethanol (25 ml) and continuously stirred for 3–5 hours at room temperature. A solid was separated out, which was water quenched (40 ml), filtered probably and then washed with water (40 ml). Finally,

**Table 2.** MIC ( $\mu\text{g/ml}$ ) and MBC performance of compounds (**6a-f**) against bacterial strains.

Compounds		Bacterial strains			
		B. cereus	E. coli	S. aureus	P. aeruginosa
6a	MIC	33.7	47.8	49.3	45.4
	MBC	67.5	95.6	97.8	99.8
6b	MIC	32.4	45.7	47.3	43.4
	MBC	65.7	93.5	86.7	97.9
6c	MIC	49.5	50.4	46.4	45.4
	MBC	98.3	100.3	99.8	99.8
6d	MIC	39.9	55.5	50.8	48.5
	MBC	78.9	112.4	101.9	88.7
6e	MIC	26.4	42.5	38.7	37.6
	MBC	43.8	84.3	75.3	75.3
6f	MIC	22.4	39.6	42.4	41.5
	MBC	45.8	78.3	85.9	93.1
Ampicillin		1.18	2.33	2.50	2.12

**Table 3.** MIC ( $\mu\text{g/ml}$ ) and MFC performance of compounds (**6a–f**) against fungal strains.

Compounds	Fungal strains				
	<i>A. niger</i>	<i>A. flavus</i>	<i>R. stolonifer</i>	<i>F. oxysporum</i>	
6a	MIC	55.2	57.3	49.8	58.3
	MFC	113.4	114.6	99.6	116.6
6b	MIC	53.6	55.7	45.3	55.8
	MFC	110.8	111.4	90.7	110.6
6c	MIC	59.5	58.2	60.2	59.1
	MFC	110.5	109.6	120.5	109.4
6d	MIC	60.5	62.5	65.5	63.2
	MFC	120.8	121.5	124.5	121.5
6e	MIC	51.6	47.3	39.5	53.5
	MFC	103.2	94.6	79	107.1
6f	MIC	49.6	52.3	42.7	48.3
	MFC	98.4	104.6	95.4	96.6
Flucanazole		2.33	1.16	4.67	2.82



**Figure 4.** Compound (**6e**) interacts with 1kzn by binding with two amino acids HIS116 through hydrogen bond. (1) Enfolded of molecules (**6e**) in the active site 1kzn complexes. (2) Hydrogen bond interaction of molecule ligand (**6e**) with 1kzn. (3) The 2D interaction analysis of (**6e**) with 1kzn. (4) Ribbon models of 1kzn and ligand molecule (**6e**).

the solid has been dried under the vacuum and recrystallized with ethanol to obtain compound (**4**).

#### (4-Chloro-phenoxy)-acetic acid hydrazide (**4**)

Yield 78%; M.P. 154°C–156°C; FT-IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1,670 (amide, C=O), 3,122–3,220 (NH-NH<sub>2</sub>); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.33 (s, 2H, NH<sub>2</sub>), 4.70 (s, 2H, OCH<sub>2</sub>), 6.80–7.41 (m, 4H, Ar-H), 9.80 (s, 1H, NH); LC-MS m/z 200 [M<sup>+</sup>], 202 [M<sup>+</sup>] (75), 128 (100), 184 (28), 186 (12), 170 (6). Anal. Calcd. for C<sub>8</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>2</sub> (200): C, 47.88; H, 4.50; N, 13.95. Found: C, 47.82%; H, 4.44%; N, 13.90%.

#### General synthetic procedure for benzoic acid N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides (**6a–f**)

To the compound (**4**), in dry DCM, (15 ml), substituted acids (**5a–f**) were added, followed by the addition of the of lutidine (1.2 vol.), at 25°C–30°C. The reaction mixture was stirred at the same temperature for 20 minutes. The reaction was cooled to 0°C–5°C by using an ice bath, TBTU (0.002 mol) was added

over a period of 25 minutes while maintaining the temperature condition below than 5°C. The reaction was stirred overnight and in during TLC used for monitoring the progress with hexane: ethyl acetate (3:1) system. Then the reaction mixture was diluted with (35 ml) of DCM and treated with 10% of sodium bicarbonate solution (35 ml). The organic layer was washed with water (35 ml), dried over anhydrous sodium sulfate and concentrated to yield compounds (**6a–f**).

#### N-(4-chloro-2-nitro)benzoyl N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (**6a**)

Yield 65%; M.P. 176°C–178°C; FT-IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1,645 (amide, C=O), 3,125–3,220 (NH-NH); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.64 (s, 2H, OCH<sub>2</sub>), 6.98–8.20 (m, 7H, Ar-H), 10.50 (s, 1H, NH), 10.71 (s, 1H, NH); LC-MS m/z 384 [M<sup>+</sup>], 386 [M<sup>+</sup>], 388 [M<sup>+</sup>] (100), 128 (6), 170 (35), 335 (35). Anal. Calcd. for C<sub>15</sub>H<sub>11</sub>C<sub>12</sub>N<sub>3</sub>O<sub>5</sub> (384): C, 46.92; H, 2.89; N, 10.94. Found: C, 46.85%; H, 2.80%; N, 10.90%.

#### N-(4-fluoro)benzoyl N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (**6b**)

Yield 80%; M.P. 173°C–175°C; FT-IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1,645 (amide, C=O), 3,125–3,220 (NH-NH); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.60 (s, 2H, OCH<sub>2</sub>), 6.95–8.15 (m, 7H, Ar-H), 10.55 (s, 1H, NH), 10.70 (s, 1H, NH); LC-MS m/z 322 [M<sup>+</sup>], 324 [M<sup>+</sup>] (100), 128 (6), 170 (35), 335 (35). Anal. Calcd. for C<sub>15</sub>H<sub>12</sub>ClFN<sub>2</sub>O<sub>3</sub> (322): C, 55.83; H, 3.75; N, 8.68. Found: C, 55.80%; H, 3.70%; N, 8.60%.

#### N-(4-methoxy)benzoyl N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (**6c**)

Yield 74%; M.P. 170°C–172°C; FT-IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1,640 (amide, C=O), 3,125–3,220 (NH-NH); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.10 (s, 3H, OCH<sub>3</sub>), 4.65 (s, 2H, OCH<sub>2</sub>), 6.90–8.19 (m, 8H, Ar-H), 10.50 (s, 1H, NH), 10.75 (s, 1H, NH); LC-MS m/z 334 [M<sup>+</sup>], 336 [M<sup>+</sup>] (100), 128 (6), 170 (35), 335 (35). Anal. Calcd. for C<sub>16</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>4</sub> (334): C, 57.41; H, 4.52; N, 8.37. Found: C, 57.35%; H, 4.50%; N, 8.30%.

*N*-(2,4,6-trimethyl)benzoyl- *N'*-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (6d)

Yield 74%; M.P. 180°C–182°C; FT-IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1,640 (amide, C=O), 3,125–3,220 (NH-NH); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.66 (s, 9H, 3CH<sub>3</sub>), 4.60 (s, 2H, OCH<sub>2</sub>), 6.95–8.25 (m, 6H, Ar-H), 10.50 (s, 1H, NH), 10.70 (s, 1H, NH); LC-MS m/z 346 [M<sup>+</sup>], 348 [M<sup>+</sup>+2] (100), 128 (6), 170 (35), 335 (35). Anal. Calcd. for C<sub>18</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>3</sub> (346): C, 62.34; H, 5.52; N, 8.08. Found: C, 62.30%; H, 5.50%; N, 8.00%.

*N*-(3,4-dichloro)benzoyl-*N'*-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (6e)

Yield 65%; M.P. 173°C–175°C; FT-IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1,640 (amide, C=O), 3,125–3,220 (NH-NH); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.62 (s, 2H, OCH<sub>2</sub>), 6.95–8.20 (m, 7H, Ar-H), 10.54 (s, 1H, NH), 10.75 (s, 1H, NH); LC-MS m/z 373 [M<sup>+</sup>], 375 [M + 2], 377 [M + 4], 379 [M + 6] (100), 128 (6), 170 (35), 335 (35). Anal. Calcd. for C<sub>15</sub>H<sub>11</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (373): C, 48.22; H, 2.97; N, 7.50. Found: C, 48.20%; H, 2.92%; N, 7.45%.

*N*-(2,3,4,5-Tetrafluoro)benzoyl- *N'*-[2-(4-chloro-phenoxy)-acetyl]-hydrazide (6f)

Yield 75%; B.P. 182°C; FT-IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 1,640 (amide, C=O), 3,125–3,220 (NH-NH); <sup>1</sup>HNMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.66 (s, 2H, OCH<sub>2</sub>), 6.92–8.22 (m, 5H, Ar-H), 10.52 (s, 1H, NH), 10.70 (s, 1H, NH); LC-MS m/z 376 [M<sup>+</sup>], 378 [M + 2] (100), 128 (6), 170 (35), 335 (35). Anal. Calcd. for C<sub>15</sub>H<sub>9</sub>ClF<sub>4</sub>N<sub>2</sub>O<sub>3</sub> (376): C, 47.83; H, 2.41; N, 7.44. Found: C, 47.80%; H, 2.40%; N, 7.40%.

**Biological study***Maintenance, culture, and preservation of the microorganisms*

Cultures in the experimental parts of bacteria and fungi were obtained from the Department of Microbiology, Maharani College, Mysore University. Preparation appropriate culture media is one of the basics to study them. Maintained pure bacterial cultures were done on the nutrient agar medium and other fungal cultures were done on potato dextrose agar (PDA) medium. Further maintained were by sub-culturing all stock cultures regularly on the same of medium and incubated at 25°C–30°C for 7–8 days and then stored at 4°C before to use in experiments.

*Media preparation and its sterilization*

Anti-microbial susceptibility has been evaluated in Petri dishes by using agar well diffusion methods on solid (Agar—Agar) media (Yehia *et al.*, 2013). Nutrient agar (NA) (34 g/l) and PDA (34 g/l) were used for the bacterial assay to develop the surface of the colony growth. The MBC, MFC and MIC, values were evaluated using the serial microdilution assays. Suspension culture, for bacterial cells growth, was done by preparing 2.25% Lauria Broth (w/v), and for the fungus cells growth, 2.45% (w/v) Potato dextrose broth (PDB) was taken for an estimate. Sterilized all the prepared media by autoclaving at (122°C) for 25 minutes.

*Microbiological screening*

Agar well diffusion method was used to evaluation anti-microbial activities of different compounds, and MIC (Nwakanma *et al.*, 2016).

*Agar well diffusion method*

Agar well—diffusion method has been used to evaluate anti-microbial activity. Both the NA and PDA plates have been swabbed (sterile cotton swabs) with a 7 hours old—broth culture of the various different bacteria and fungi. Wells (10 mm diameter as well as about 2 cm in a part) have been probably made in all of these plates using sterile cork borer. Stock solution was prepared for each six compounds at a concentration of 1mg/ml. Approximately 100  $\mu$ l of different of compound concentrations were added to the wells with a sterile syringe and allowed to spread for 2 hours at 25°C. Control experiments were arranged that included inoculums without compounds. The plates have been incubated for bacterial pathogens at 38°C for 198–24 hours and for fungal pathogens at 27°C for 45 hours. Zone inhibition (mm) diameter was measured and the activity index had been calculated. Test was constantly repeated for three times (Triplicates), and the measurements were taken in three totally different fixed directions for each replicate and the average values given were recorded (Sokovic *et al.*, 2008).

*Preparation of inoculums*

## Antibacterial activity test:

The anti-bacterial assays were performed using the micro-dilution test to determine the anti-bacterial activity of organic compounds evaluated against some of the pathogenic bacteria. adjusted of bacterial suspensions to a concentration of  $1.0 \times 10^7$  CFU/ml was with sterile saline. The inocula were prepared, vacuum packaged and stored until use at 4°C. Inocula dilutions have been grown on a solid type of media to confirm the absence of toxic effects, contamination and to check the validity of the inoculum buffer. All the experiments had all been duplicated and repeated three times constantly (Mimica-Dukić *et al.*, 2003).

## Test for anti-fungal activity:

A modified micro-dilution technique has been used to thoroughly investigate the compounds as anti-fungal activity. sterile of 0.85% saline containing 0.1% between 80 (v/v) was used to wash the fungal spores from the plates agar surface. The concentration of sterile saline  $1.0-10^7$  was adjusted to spore suspension in a final volume of the 100  $\mu$ l per well. The inocula was stored to be used again at 4°C. Serial dilutions of that same inoculum were cultured on solid potato dextrose agar to check both the validity of inoculum and the absence of contamination (Sen and Batra, 2012).

## Determination of MIC:

A serial dilution method was used to perform the MBCs, MICs, and MFCs by using 96-well microtiter plates. Both for bacterial culture and fungus, potato dextrose broth medium with particular inoculum was used for the different six targeted compounds solution (1 mg/ml) with Luria broth for 72 hours, microplates were incubated at 25°C, respectively. In the binocular microscope, MICs were defined as the lowest concentrations without visible growth (Omar *et al.*, 2010).

## Determination of MBC:

By serial subcultivation of 2  $\mu$ l into microtitre plates containing 100  $\mu$ l of broth per well, MBCs were determined

for further incubation with 75 hours. MBCs were defined at the lowest concentration without visible growth which, indicating the original inoculum killed with 99%. Microplate reader (Perlong, ENM8602) at 650 nm used to determine the of each well optical density and compared with bacterial ampicillin standards (Hi-media lab, India) as the positive control. All experiments have been duplicated and repeated three times.

#### Determination of MFC:

By serial subcultivation of 2 µl into Microtiter plates with 100 µl of broth per well, the fungicidal concentrations (MFCs) were evaluated for the further incubated 75 hours at 25°C. MFC indicating 99.5% killing of the original inoculum described as the lowest concentration with no visible growth, fluconazole has been used as a positive control for fungus with (1–3,000 µg/ml). All experiments have been duplicated and repeated three times.

#### Molecular docking studies

In order to predict the interaction of synthesized compounds with the binding sites of DNA-gyrase, molecular docking studies were performed. The crystal structure of the enzyme (PDB code 1KZN) with resolution 4 Å was chosen as the protein model for the current study. The structure of ligand was optimized using the BioChemUltra2010 as per the reported method. Molecules and parameters used by Auto Dock Tools were prepared before submitting for docking analysis. Polar hydrogen atoms have been added while non-polar hydrogen atoms were merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotatable ligand bonds, defined by the program's default, were allowed to rotate throughout the automated docking system and will then prepared protein and ligand structures were calculating of energy grid maps after saved in the PDBQT suitable format. A grid box size of 44 × 44 × 44 Å points with a grid spacing of 0.377 Å was considered. Lamarckian genetic algorithm program with an adaptive whole method search in the Auto Dock was chosen to calculate the conformers of different ligand (Yasser *et al.*, 2018).

#### CONCLUSION

In conclusion, six novel hydrazide derivatives, N-benzoyl-N'-[2-(4-chloro-phenoxy)-acetyl]-hydrazides (**6a–f**), were synthesis, and evaluated for their anti-microbial action against four bacterial and four fungal strains. From the anti-microbial data, it was concluded that compounds (**6e**) bearing two chloro group and (**6f**) with four fluoro group, at 3, 4 and 2, 3, 4, 5 positions, respectively in ring **B** were highly active against both of bacterial and fungal strains. Moreover, the two potent compounds (**6e**, **6f**), showed good potential of MIC, MBC, and MFC among of all synthesis scheme. Furthermore, the *in silico* study results confirmed that the potent compound (**6e**), has good binding energy with exact pocket side to the specific protein and this result was matched to *in vitro* study.

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