



## Original article

## Hantzsch reaction: Synthesis and characterization of some new 1,4-dihydropyridine derivatives as potent antimicrobial and antioxidant agents

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## ABSTRACT

In the present study two new series of Hantzsch 1,4-dihydropyridine derivatives (1,4-DHPs) containing substituted pyrazole moiety (**4a–f** and **5a–f**) were synthesized by the reaction of 3-aryl-1H-pyrazole-4-carbaldehydes with 1,3-dicarbonylcompounds (ethylacetoacetate and methylacetoacetate) and ammonium acetate. The newly synthesized compounds were characterized by IR, NMR, mass spectral study and also by C, H, N analyses. New compounds were screened for their antimicrobial activity by well plate method (zone of inhibition). Antioxidant studies of the synthesized compounds were also performed by measuring the DPPH radical scavenging assay. Compounds **4c**, **4e** and **4f** were found to be potent antibacterial and antioxidant agents. The acute oral toxicity study for the compounds **4c**, **4e** and **4f** were carried out and the experimental studies revealed that compounds **4c** and **4e** is safe up to 3000 mg/kg and no death of animals were recorded. However in compound **4f**, we found mortality above 2000 mg and also significant behavioral changes in experimental animals.

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## 1. Introduction

In recent decades, multicomponent reactions (MCR's) have gained wide applicability in the field of synthetic organic chemistry as they increase the efficiency of the reaction and decrease the number of laboratory operations along with quantities of solvent and chemicals used. These methods also considerably reduce the reaction time and facilitate the yield of products than the normal multiple step methods. One-pot, four-component synthesis of symmetrically substituted 1,4-dihydropyridines were first reported by Arthur Hantzsch in 1882 [1]. Hantzsch 1,4-dihydropyridines (1,4-DHPs) and their derivatives are an important class of bioactive molecules in the pharmaceutical field [2]. They possess anti-inflammatory, anti-microbial [3], anti-oxidant, antiulcer activities [4]. DHPs are commercially used as calcium channel blockers for the treatment of cardiovascular diseases, including hypertension [5]. Recently, the synthesis of DHPs with respect to Multidrug Resistance (MDR) reversal in tumor cell gave a new dimension to their applications [6,7]. In addition, 1,4-DHP class of compounds are excellent starting synthons for development of antitubercular

agents [8,9]. Oxidative aromatization reactions of DHPs are taking place in biological systems in presence of certain enzymes. The nitrogen heterocycles thus prepared by Hantzsch method are of great importance because of their role in biological systems. They have been served as model compounds for the NAD-NAPH biological redox systems [10–12].

Recently, antibiotic-resistant microbes are making their inexorable march and medicinal chemists have now realized that the discovery of more powerful antibiotics is not the only answer to this threat. But, a real need exists in searching a novel antimicrobial that expresses antimicrobial properties, possibly acting through mechanisms different from those of existing drugs. In this context, it is very essential to successfully develop novel, efficient antimicrobial agents with clinically unexploited mode of action.

Further, pyrazole derivatives have showed significant biological activities, such as anti-microbial [13], analgesic [14], anti-inflammatory [15] and, anticancer [16]. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituents. Keeping in view of this and in continuation of our search on biologically potent molecules [17–21], we hereby report the synthesis of some new 1,4-dihydropyridine derivatives containing pyrazole nucleus. These compounds were evaluated for their antimicrobial and antioxidant properties.

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## 2. Results and discussion

### 2.1. Chemistry

The schematic representation of the new 1,4-dihydropyridine derivatives (**4a–f** and **5a–f**) has been presented in Scheme 1. 3-Substituted-1*H*-pyrazole-4-carbaldehydes (**3a–f**) were synthesized by the Vilsmyer Haack reaction of semicarbazones (**2a–f**) [22]. Refluxing 3-substituted-1*H*-pyrazole-4-carbaldehydes (**3a–f**), 1,3-dicarbonyl compounds (ethylacetoacetate and methylacetoacetate) and ammonium acetate in ethanol resulted in the target compounds (**4a–f** and **5a–f**) via one-pot multicomponent reaction. The proposed mechanism for the formation of dihydropyridine derivatives has been presented in Scheme 2.

Structures of the synthesized compounds **4a–f** and **5a–f** were confirmed by recording their IR, NMR, mass spectra and C, H, N elemental analyses. All compounds were characterized after recrystallization from appropriate solvents. IR spectrum of compound **4a** showed absorption at  $3243\text{ cm}^{-1}$  which is due to the NH stretching. Band at  $1667\text{ cm}^{-1}$  is due to C=O stretch. Similarly, band at  $1608\text{ cm}^{-1}$  is due to C=N group confirmed the structure. The  $^1\text{H}$  NMR spectrum of **4a** showed a triplet at  $\delta$  0.96 is due to  $\text{CH}_3$  protons of  $\text{CH}_2\text{--CH}_3$  group. A singlet at  $\delta$  2.28 is due to  $\text{CH}_3$  protons. A quartet appeared at  $\delta$  3.87 which is due to  $\text{CH}_2$  protons of  $\text{O--CH}_2\text{--CH}_3$  group. Pyridine-4H appeared as a singlet at  $\delta$  5.04. Two doublets at  $\delta$  7.02 and  $\delta$  7.76 are due to aromatic protons of *p*-anisyl moiety. Pyrazole-5H proton appeared as a singlet at  $\delta$  7.25.

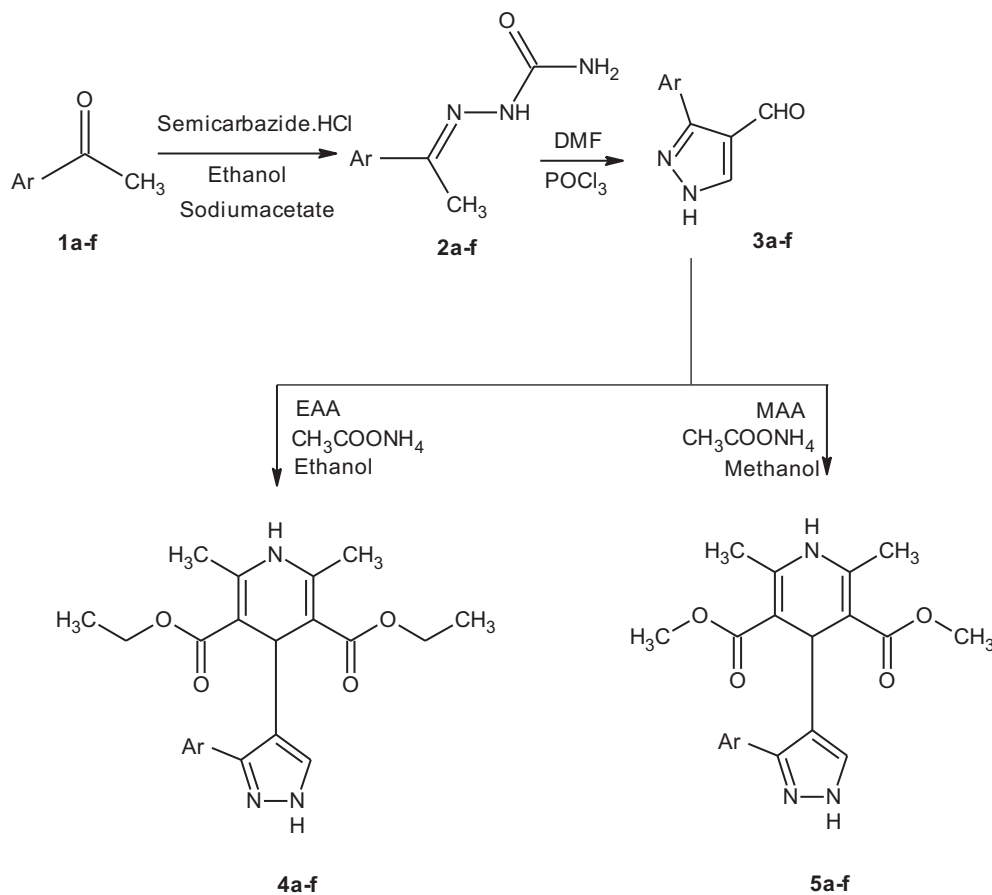
NH protons of dihydropyridine appeared as a singlet at  $\delta$  8.56. A singlet appeared at  $\delta$  12.58 is due to pyrazole-NH further confirmed the structure. The mass spectrum of **4a** showed molecular ion peak at  $m/z = 424.1$  ( $m-1$ ), which is in agreement with the molecular formula  $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_5$ . Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization is provided in Tables 1 and 2.

### 2.2. Antimicrobial studies

#### 2.2.1. Antibacterial studies

The *in vitro* antibacterial activity of newly synthesized compounds **4a–f** and **5a–f** were determined by well plate method in Mullere Hinton Agar [23,24]. In this work, *Escherichia coli* ATCC 25922 (Gram-negative), *Staphylococcus aureus* ATCC 25923 (Gram-positive) and *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative) were selected due to their infectious nature. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 1, 0.5 and 0.25 mg/mL.

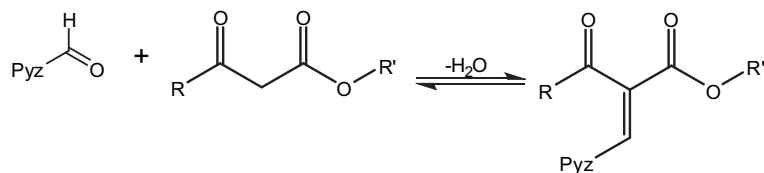
The antibacterial screening revealed that, some of the tested compounds showed good inhibition against various tested microbial strains. The result indicated that among the tested compounds, **4c** and **4f** showed excellent activity against all the tested microbial strains *E. coli*, *S. aureus* and *P. aeruginosa* at concentrations of 1, 0.5 and 0.25 mg/mL compared to standard drug streptomycin. Compound **4d** showed good antibacterial activity against *E. coli* and *P. aeruginosa* as compared to the standard drug. **4e** showed



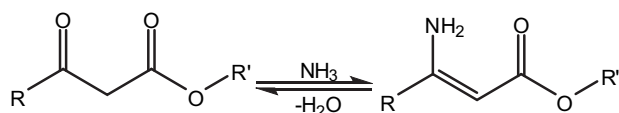
Where Ar = 2,4-Dichlorophenyl, 4-Thioanisyl, 2,5-Dichlorothiophene, Biphenyl, 4-Anisyl, 4-Chlorophenyl

Scheme 1. Synthetic route for the compounds **4a–f** and **5a–f**.

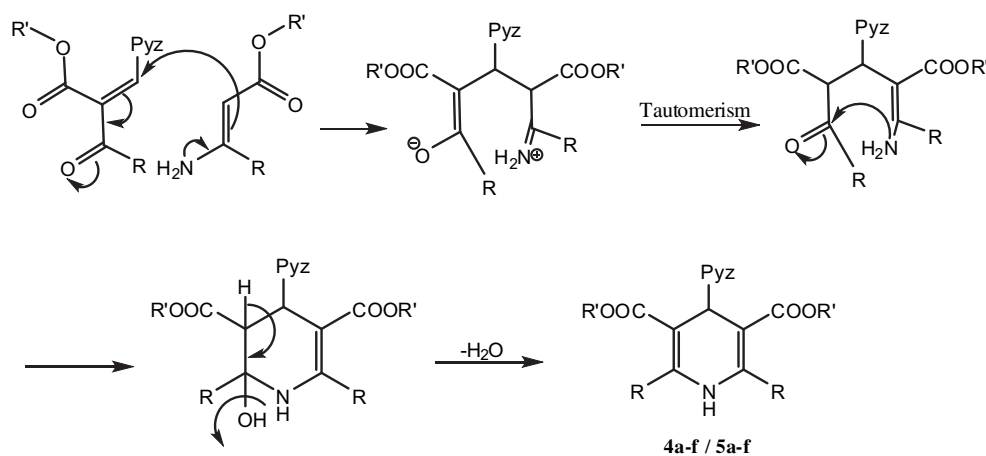
**Step-1:** The reaction can be visualized as proceeding through a Knoevenagel Condensation product as a key intermediate



**Step-2:** Formation of enamine-type intermediate



**Step-3:** Condensation between the two fragments gives the dihydropyridine derivative.



Pyz = Substituted pyrazoles, R = CH<sub>3</sub>, R<sup>1</sup> = CH<sub>3</sub> or C<sub>2</sub>H<sub>5</sub>

**Scheme 2.** Proposed reaction mechanism for the formation of substituted dihydropyridines.

excellent activity as that of standard, against *S. aureus* and *P. aeruginosa* at all concentrations. Compounds **4a** and **4b** showed moderately good anti-microbial activity against all the tested microbial strains. The remaining compounds have showed less activity against all of the three tested bacterial strains compared to standard, streptomycin. The antibacterial results were summarized in Table 3.

Compound **4c** has thioanisyl moiety on pyrazole ring, which is accounted for the enhanced antibacterial activity. Compound **4f** has 4-chlorophenyl substituent, **4d** has 2,5-dichlorothiophene and **4e** has biphenyl respectively on pyrazole ring, which is accounted for the enhanced activity of the compounds. Similarly compounds **4a** and **4b** have anisyl and 2,4-dichlorophenyl substituents, which is responsible for their biological activity.

**Table 1**  
Characterization data of the compounds **3a–f**.

Comp. No.	Ar	Molecular Formula (Mol. wt.)	Yield (%)	M.p. (°C)	Color
<b>3a</b>	2,4-Dichlorophenyl	C <sub>10</sub> H <sub>6</sub> Cl <sub>2</sub> N <sub>2</sub> O (241.07)	87	114–116	Off white
<b>3b</b>	4-Thioanisyl	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> OS (218.27)	91	148–150	Off white
<b>3c</b>	2,5-Dichloro thiophene	C <sub>8</sub> H <sub>4</sub> Cl <sub>2</sub> N <sub>2</sub> OS (247.10)	90	138–140	Off white
<b>3d</b>	Biphenyl	C <sub>16</sub> H <sub>12</sub> N <sub>2</sub> O (248.27)	88	178–180	Yellow
<b>3e</b>	4-Anisyl	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub> (202.20)	86	162–164	Off white
<b>3f</b>	4-Chlorophenyl	C <sub>10</sub> H <sub>7</sub> ClN <sub>2</sub> O (206.62)	87	142–144	Off white

### 2.2.2. Antifungal studies

Newly synthesized compounds **4a–f** and **5a–f** were also screened for their antifungal activity against *Aspergillus flavus* MTCC 3306, *Chrysosporium keratinophilum* MTCC 2827 and *Candida albicans* MTCC 3017, because of their infectious nature. The compounds were dissolved in DMSO and antimicrobial activity was determined by well plate method [25,26] at concentrations of 1, 0.5 and 0.25 mg/mL.

**Table 2**  
Characterization data of the compounds **4a–f** and **5a–f**.

Comp. No.	Ar	Molecular Formula (Mol. wt.)	Yield (%)	M.p. (°C)	Color
<b>4a</b>	4-Anisyl	C <sub>23</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub> (425.47)	76	180–182	Off white
<b>4b</b>	2,4-Dichlorophenyl	C <sub>22</sub> H <sub>23</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>4</sub> (464.34)	73	184–186	Off white
<b>4c</b>	4-Thioanisyl	C <sub>23</sub> H <sub>27</sub> N <sub>3</sub> O <sub>4</sub> S (441.54)	82	162–164	Off white
<b>4d</b>	2,5-Dichloro thiophene	C <sub>20</sub> H <sub>21</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>4</sub> S (470.36)	69	169–171	Off white
<b>4e</b>	Biphenyl	C <sub>28</sub> H <sub>29</sub> N <sub>3</sub> O <sub>4</sub> (471.57)	74	192–194	Yellow
<b>4f</b>	4-Chlorophenyl	C <sub>22</sub> H <sub>24</sub> ClN <sub>3</sub> O <sub>4</sub> (429.89)	67	186–188	Off white
<b>5a</b>	2,4-Dichlorophenyl	C <sub>20</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>4</sub> (436.28)	77	202–204	Off white
<b>5b</b>	4-Thioanisyl	C <sub>21</sub> H <sub>23</sub> N <sub>3</sub> O <sub>4</sub> S (413.49)	84	194–196	Yellow
<b>5c</b>	2,5-Dichloro thiophene	C <sub>18</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>4</sub> S (442.31)	80	214–216	Yellow
<b>5d</b>	Biphenyl	C <sub>26</sub> H <sub>25</sub> N <sub>3</sub> O <sub>4</sub> (443.49)	78	246–248	Off white
<b>5e</b>	4-Anisyl	C <sub>21</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub> (397.42)	81	162–164	Off white
<b>5f</b>	4-Chlorophenyl	C <sub>20</sub> H <sub>20</sub> ClN <sub>3</sub> O <sub>4</sub> (401.84)	75	252–254	Off white

**Table 3**  
Antibacterial activity of the compounds **4a–f** and **5a–f** (Zone of inhibition in mm.).

Comp. No.	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>			<i>Pseudomonas aeruginosa</i>		
	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25
Standard Streptomycin	16 ± 0.01	10 ± 0.02	8 ± 0.02	15 ± 0.02	10 ± 0.01	9 ± 0.02	16 ± 9.02	13 ± 0.01	11 ± 0.02
<b>4a</b>	12 ± 0.01	08 ± 0.02	05 ± 0.01	13 ± 0.02	08 ± 0.01	06 ± 0.02	10 ± 0.01	07 ± 0.02	05 ± 0.01
<b>4b</b>	06 ± 0.02	04 ± 0.01	02 ± 0.02	11 ± 0.01	07 ± 0.02	05 ± 0.01	12 ± 0.01	09 ± 0.02	07 ± 0.01
<b>4c</b>	17 ± 0.02	12 ± 0.01	09 ± 0.01	16 ± 0.01	12 ± 0.02	10 ± 0.01	14 ± 0.01	11 ± 0.01	08 ± 0.01
<b>4d</b>	16 ± 0.02	10 ± 0.01	08 ± 0.01	13 ± 0.01	09 ± 0.02	08 ± 0.01	15 ± 0.02	12 ± 0.01	10 ± 0.01
<b>4e</b>	13 ± 0.02	10 ± 0.01	07 ± 0.02	16 ± 0.01	12 ± 0.01	10 ± 0.02	17 ± 0.02	13 ± 0.01	11 ± 0.02
<b>4f</b>	17 ± 0.01	12 ± 0.01	10 ± 0.02	15 ± 0.01	10 ± 0.02	08 ± 0.01	16 ± 0.01	13 ± 0.01	10 ± 0.02
<b>5a</b>	06 ± 0.01	03 ± 1	02 ± 0.01	06 ± 0.01	03 ± 0.01	02 ± 0.01	08 ± 0.02	04 ± 0.01	02 ± 0.01
<b>5b</b>	04 ± 0.01	02 ± 0.01	01 ± 0.01	06 ± 0.02	03 ± 0.02	02 ± 0.01	06 ± 0.02	04 ± 0.02	03 ± 0.01
<b>5c</b>	10 ± 0.01	07 ± 0.02	05 ± 0.01	10 ± 0.02	08 ± 0.02	04 ± 0.01	07 ± 0.01	03 ± 0.02	01 ± 0.01
<b>5d</b>	13 ± 0.02	10 ± 0.01	08 ± 0.02	09 ± 0.01	07 ± 0.02	05 ± 0.01	10 ± 0.02	08 ± 0.02	07 ± 0.01
<b>5e</b>	07 ± 0.02	04 ± 0.02	02 ± 0.01	06 ± 0.02	04 ± 0.02	02 ± 0.02	07 ± 0.01	04 ± 0.01	02 ± 0.02
<b>5f</b>	10 ± 0.01	08 ± 0.01	05 ± 0.02	08 ± 0.01	06 ± 0.02	03 ± 0.01	09 ± 0.01	07 ± 0.02	04 ± 0.01

All the compounds (**4a–f** and **5a–f**) showed less antifungal activity against all the tested micro organisms compared to standard drug, fluconazole. Results of antifungal studies have been presented in Table 4.

### 2.3. Antioxidant studies: DPPH radical scavenging assay

The free radical scavenging activity of test samples **4a–f** and **5a–f** was measured by DPPH according to Brand-Williams et al. [27]. All compounds have exhibited free radical scavenging capacity by comparison with the standard Butylated Hydroxytoluene (BHT). DPPH assay were carried out for compounds **4a–f** and **5a–f** at 100 µM concentration. The antioxidant activity may be, one possible mechanism responsible for the organ protective effects of 1,4-dihydropyridine calcium channel blockers [28,29]. Nifedipine is a well known 1,4-dihydropyridine derivative [30].

Among the tested compounds (**4a–f** and **5a–f**), compounds **4c**, **4e** and **4f** showed significant amount of DPPH activity (>60%). Remaining were non significant compared to the standard BHT. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitutions and results are presented in Table 5.

### 2.4. Acute toxicity and behavioral studies

The acute oral toxicity study for the test compounds (compounds **4c**, **4e** and **4f**) was carried out by following the OECD guidelines [31,32]. The experimental studies revealed that compounds **4c** and **4e** are safe up to 3000 mg/kg and no deaths of animals were recorded. Further, no significant behavioral changes

were observed in experimental animals. But in compound **4f**, we found mortality in above 2000 mg and also significant behavioral changes were observed in experimental animals.

### 3. Conclusion

Two series of new substituted Hantzsch 1,4-dihydropyridine derivatives (1,4-DHPs) containing substituted pyrazole moiety (**4a–f** and **5a–f**) were synthesized in reasonably good yields. They were characterized by spectral studies and elemental analyses. All the newly synthesized compounds were screened for antimicrobial and antioxidant activity.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, it showed varied biological activity. Compounds **4c** and **4f** have showed excellent antibacterial activity. Among the two series, **4a–f** which contains ethyl ester moiety on dihydropyridine ring showed good activity as compared to the second series, **5a–f** which consists of a methyl ester group. Antifungal studies reveal that all the tested compounds were less active compared to the standard drug. From the antimicrobial results we can conclude that, synthesized compounds are specific antibacterial agents. A combination of two different heterocyclic systems namely pyrazole and 1,4-dihydropyridine has enhanced the pharmacological effect and hence they are ideally suited for further modifications to obtain more efficacious antibacterial compounds.

The free radical scavenging activity of test samples **4a–f** and **5a–f** was measured by DPPH. The data reported herein indicates that compounds **4c**, **4e** and **4f** showed significant DPPH activity (>60%) and the remaining compounds were non significant

**Table 4**  
Antifungal activity of the compounds **4a–f** and **5a–f** (Zone of inhibition in mm.).

Comp. No.	<i>Aspergillus Flavus</i>			<i>Chrysosporium Keratinophilum</i>			<i>Candida Albicans</i>		
	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25
Standard Fluconazole	13 ± 0.01	11 ± 0.02	10 ± 0.01	17 ± 0.01	15 ± 0.02	14 ± 0.01	22 ± 0.01	21 ± 0.02	20 ± 0.02
<b>4a</b>	02 ± 0.01	01 ± 0.01	00	03 ± 0.01	02 ± 0.01	00	03 ± 0.01	01 ± 0.01	02 ± 0.01
<b>4b</b>	03 ± 0.01	02 ± 0.01	02 ± 0.01	04 ± 0.01	02 ± 0.01	01 ± 0.01	04 ± 0.01	01 ± 0.01	01 ± 0.01
<b>4c</b>	00	00	00	00	00	00	00	00	00
<b>4d</b>	04 ± 0.02	03 ± 0.01	03 ± 0.01	05 ± 0.01	02 ± 0.01	02 ± 0.01	05 ± 0.01	02 ± 0.01	02 ± 0.01
<b>4e</b>	05 ± 0.01	02 ± 0.01	01 ± 0.01	03 ± 0.01	00 ± 0.01	00	06 ± 0.02	04 ± 0.01	02 ± 0.01
<b>4f</b>	02 ± 0.01	00	00	03 ± 0.01	00	00	04 ± 0.01	02 ± 0.01	00
<b>5a</b>	03 ± 0.01	02 ± 0.01	00	05 ± 0.01	01 ± 0.01	00	03 ± 0.01	00	00
<b>5b</b>	00	00	00	00	00	00	00	00	00
<b>5c</b>	03 ± 0.01	02 ± 0.01	00	04 ± 0.02	02 ± 0.01	01 ± 0.01	03 ± 0.01	02 ± 0.01	00
<b>5d</b>	05 ± 0.02	04 ± 0.01	02 ± 0.01	03 ± 0.01	02 ± 0.01	00	00	00	0
<b>5e</b>	00	00	00	00	00	00	00	00	00
<b>5f</b>	06 ± 0.02	03 ± 0.01	01 ± 0.01	05 ± 0.02	04 ± 0.01	02 ± 0.01	03 ± 0.01	01 ± 0.01	00

**Table 5**  
DPPH radical scavenging activity of synthesized compounds **4a–f** and **5a–f**.

Compound No.	DPPH Assay in %
<b>4a</b>	30.3
<b>4b</b>	29.2
<b>4c</b>	61.1
<b>4d</b>	32.0
<b>4e</b>	65.3
<b>4f</b>	62.3
<b>5a</b>	35.2
<b>5b</b>	32.2
<b>5c</b>	30.3
<b>5d</b>	25.3
<b>5e</b>	30.2
<b>5f</b>	29.4
BHT	72.42

compared to the standard BHT. The acute oral toxicity study for the compounds **4c**, **4e** and **4f** were carried out and the experimental studies revealed that compounds **4c** and **4e** is safe up to 3000 mg/kg and no death of animals were recorded. But in compound **4f** we found mortality in above 2000 mg and also significant behavioral changes were observed in experimental animals.

## 4. Experimental

### 4.1. Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer.  $^1\text{H}$  NMR spectra were recorded (DMSO- $d_6$ ) on a Bruker (400 MHz, 300 MHz) using TMS as internal standard. Chemical shift values are given in  $\delta$  (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

### 4.2. General procedure for the synthesis of (2E)-2-(1-arylethylidene)hydrazinecarboxamide (**2a–f**)

A solution of semicarbazide hydrochloride (1.07 mmol) in 20 mL of water was added drop wise to a round bottom flask containing mixture of substituted carbonyl compounds **1a–f** (1.0 mmol), sodium acetate (1.3 mmol) and ethanol (20 mL). The reaction mixture was stirred at 80 °C for 8 h. After the reaction completion, the separated solids were filtered, washed with water and dried. The crude products (**2a–f**) as such taken for next stage preparation without further purification.

### 4.3. General procedure for the synthesis of 3-substituted-1H-pyrazole-4-carbaldehydes (**3a–f**)

3-Substituted-1H-pyrazole-4-carbaldehydes (**3a–f**) were synthesized by Vilsmayer–Haack reaction. To an ice cold solution of (2E)-2-(1-arylethylidene)hydrazinecarboxamide (**2a–f**) (0.1 mmol) in DMF (20 mL), POCl<sub>3</sub> (8 mL) was added drop wise. After the addition, the reaction mixture was stirred for 30 min at ambient temperature and then stirred at 60–65 °C for 6 h. The reaction mixture was quenched into ice cold water and pH adjusted to 7 using 25% sodium hydroxide solution. The solids thus precipitated were filtered and dried. Crude product was recrystallized from ethyl acetate.

#### 4.3.1. 3-(2,4-Dichlorophenyl)-1H-pyrazole-4-carbaldehyde (**3a**)

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  7.49–7.54 (m, 2H, Ar–H), 7.76 (s, 1H, Ar–H), 8.47 (s, 1H, pyrazole-5H), 9.71 (s, 1H, –CHO), 13.74 (s, 1H, pyrazole-NH);  $^{13}\text{C}$  NMR:  $\delta$  184.40, 147.59, 133.89, 133.29, 129.04, 127.28, 120.95; MS:  $m/z$  = 242.0 (M + 1); Anal. calcd. for C<sub>10</sub>H<sub>6</sub>Cl<sub>2</sub>N<sub>2</sub>O: C, 49.82; H, 2.51; N, 11.62; Found: C, 49.80; H, 2.48; N, 11.59%.

#### 4.3.2. 3-[4-(Methylsulfanyl)phenyl]-1H-pyrazole-4-carbaldehyde (**3b**)

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.29 (s, 3H, SCH<sub>3</sub>), 7.35 (m, 2H, Ar–H), 7.79 (m, 2H, Ar–H), 8.56 (s, 1H, pyrazole-5H), 9.87 (s, 1H, –CHO), 13.64 (s, 1H, pyrazole-NH);  $^{13}\text{C}$  NMR:  $\delta$  184.65, 141.54, 130.68, 128.84, 125.60, 14.35; MS:  $m/z$  = 219.2 (M + 1); Anal. calcd. for C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>OS: C, 60.53; H, 4.62; N, 12.83; Found: C, 60.50; H, 4.61; N, 12.81%.

#### 4.3.3. 3-(2,5-Dichlorothiophen-3-yl)-1H-pyrazole-4-carbaldehyde (**3c**)

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  7.35 (s, 1H, 2,5-dichlorothiophene), 8.49 (s, 1H, pyrazole-5H), 9.80 (s, 1H, –CHO), 13.69 (s, 1H, pyrazole-NH);  $^{13}\text{C}$  NMR:  $\delta$  184.43, 161.15, 128.87, 120.69; MS:  $m/z$  = 248.1 (M + 1); Anal. calcd. for C<sub>8</sub>H<sub>4</sub>Cl<sub>2</sub>N<sub>2</sub>OS: C, 38.89; H, 1.63; N, 11.34; Found: C, 38.86; H, 1.61; N, 11.31%.

### 4.4. General procedure for the synthesis of diethyl-4-(3-aryl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4a–f**)

3-(4-Substituted)-1H-pyrazole-4-carbaldehyde (1.0 mmol), ethylacetate (2.0 mmol) and ammonium acetate (1.12 mmol) in ethanol (20 mL) were refluxed for 8 h in an oil bath. After the reaction completion, reaction mixture was concentrated and poured in to crushed ice. The precipitated product was filtered, washed with water. The resulting solid was recrystallized from hot ethanol.

#### 4.4.1. Diethyl-4-[3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4a**)

IR (KBr,  $\nu_{\text{max}}$  cm<sup>-1</sup>): 3243 (N–H-str), 3066, 2975 (C–H-str), 1667 (C=O), 1608 (C=N);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  0.86 (t, 6H, CH<sub>2</sub>–CH<sub>3</sub>), 2.16 (s, 6H, CH<sub>3</sub>), 3.74 (s, 3H, OCH<sub>3</sub>), 3.97 (m, 4H, CH<sub>2</sub>), 4.99 (s, 1H, pyridine-4H), 6.98 (d, 2H,  $J$  = 8.17 Hz, Ar–H), 7.18 (s, 1H, pyrazole-5H), 7.68 (d, 2H,  $J$  = 8.28, Ar–H), 8.77 (s, 1H, pyridine-NH), 12.46 (s, 1H, pyrazole-NH);  $^{13}\text{C}$  NMR:  $\delta$  167.61, 159.19, 145.20, 129.42, 114.05, 103.17, 59.27, 55.66, 28.66, 18.65, 14.48; MS:  $m/z$  = 424.1 (M – 1); Anal. calcd. for C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub>: C, 64.93; H, 6.40; N, 9.88; Found: C, 64.90; H, 6.36; N, 9.85%.

#### 4.4.2. Diethyl-4-[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4b**)

IR (KBr,  $\nu_{\text{max}}$  cm<sup>-1</sup>): 3286 (N–H-str), 3061, 2982 (C–H-str), 1678 (C=O), 1651 (C=N);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  1.08 (t, 6H, CH<sub>2</sub>–CH<sub>3</sub>), 2.52 (s, 6H, CH<sub>3</sub>), 3.90 (m, 4H, CH<sub>2</sub>), 5.04 (s, 1H, pyridine-4H), 7.24–7.65 (m, 4H, Ar–H, pyrazole-5H), 8.47 (s, 1H, pyridine-NH), 12.59 (s, 1H, pyrazole-NH);  $^{13}\text{C}$  NMR:  $\delta$  167.40, 145.25, 135.46, 133.96, 101.39, 59.26, 28.96, 18.43, 14.64; MS:  $m/z$  = 465.3 (M + 1); Anal. calcd. for C<sub>22</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>: C, 59.91; H, 4.99; N, 9.05; Found: C, 59.89; H, 4.96; N, 9.02%.

#### 4.4.3. Diethyl-4-[3-[4-(methylsulfanyl)phenyl]-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4c**)

IR (KBr,  $\nu_{\text{max}}$  cm<sup>-1</sup>): 3213 (N–H-str), 3066, 2978 (C–H-str), 1680 (C=O), 1623 (C=N);  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  0.91 (t, 6H, CH<sub>2</sub>–CH<sub>3</sub>), 2.22 (s, 6H, CH<sub>3</sub>), 2.51 (s, 3H, SCH<sub>3</sub>), 3.96 (m, 4H, CH<sub>2</sub>),

4.88 (s, 1H, pyridine-4H), 7.22(s, 1H, pyrazole-5H), 7.35 (m, 2H, Ar-H), 7.74 (m, 2H, Ar-H), 8.83 (s, 1H, pyridine-NH), 12.60 (s, 1H, pyrazole-NH); MS:  $m/z = 442.5 (M + 1)$ ; Anal. calcd. for  $C_{23}H_{27}N_3O_4S$ : C, 62.56; H, 6.16; N, 9.52; Found: C, 62.54; H, 6.13; N, 9.50%.

#### 4.4.4. Diethyl-4-[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4d**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3240 (N-H-str), 3052, 2966 (C-H-str), 1668 (C=O), 1614 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  1.10 (t, 6H,  $\text{CH}_2\text{-CH}_3$ ), 2.50 (s, 6H,  $\text{CH}_3$ ), 3.92 (m, 4H,  $\text{CH}_2$ ), 4.84 (s, 1H, pyridine-4H), 7.22–7.36 (m, 2H, 2,5-dichlorothiophene, pyrazole-5H), 8.63 (s, 1H, pyridine-NH), 12.72 (s, 1H, pyrazole-NH); MS:  $m/z = 471.3 (M + 1)$ ; Anal. calcd. for  $C_{20}H_{21}Cl_2N_3O_4S$ : C, 51.07; H, 4.50; N, 8.93; Found: C, 51.05; H, 4.47; N, 8.90%.

#### 4.4.5. Diethyl-4-[3-(biphenyl-4-yl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4e**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3256 (N-H-str), 3064, 2927 (C-H-str), 1682 (C=O), 1625 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  1.09 (t, 6H,  $\text{CH}_2\text{-CH}_3$ ), 2.24 (s, 6H,  $\text{CH}_3$ ), 3.91 (m, 4H,  $\text{CH}_2$ ), 5.02 (s, 1H, pyridine-4H), 7.25–7.79 (m, 10H, Ar-H, pyrazole-5H), 8.83 (s, 1H, pyridine-NH), 12.69 (s, 1H, pyrazole-NH); MS:  $m/z = 472.5 (M + 1)$ ; Anal. calcd. for  $C_{28}H_{29}N_3O_4$ : C, 71.32; H, 6.20; N, 8.91; Found: C, 71.30; H, 6.17; N, 8.87%.

#### 4.4.6. Diethyl-4-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4f**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3235 (N-H-str), 3072, 2937 (C-H-str), 1671 (C=O), 1635 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  0.91 (t, 6H,  $\text{CH}_2\text{-CH}_3$ ), 2.21 (s, 6H,  $\text{CH}_3$ ), 3.90 (m, 4H,  $\text{CH}_2$ ), 5.03 (s, 1H, pyridine-4H), 7.29 (s, 1H, pyrazole-5H), 7.52 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H), 8.83 (s, 1H, pyridine-NH), 12.67 (s, 1H, pyrazole-NH); MS:  $m/z = 430.8 (M + 1)$ ; Anal. calcd. for  $C_{22}H_{24}ClN_3O_4$ : C, 61.46; H, 5.63; N, 9.77; Found: C, 61.42; H, 5.61; N, 9.74%.

#### 4.5. General procedure for the synthesis of dimethyl 4-(3-aryl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5a–f**)

3-(4-Aryl)-1H-pyrazole-4-carbaldehyde (1.0 mmol), methyl-acetoacetate (2.0 mmol) and ammonium acetate (1.12 mmol) in ethanol (20 mL) were refluxed for 8 h in an oil bath. After the reaction completion, reaction mixture was concentrated and poured in to crushed ice. The precipitated product was filtered, washed with water. The resulting solid was recrystallized from hot ethanol.

#### 4.5.1. Dimethyl 4-[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5a**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3262 (N-H-str), 3057, 2946 (C-H-str), 1692 (C=O), 1632 (C=N);  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  2.32 (s, 6H,  $\text{CH}_3$ ), 3.48 (s, 6H, O- $\text{CH}_3$ ), 4.97 (s, 1H, pyridine-4H), 7.22–7.64 (m, 4H, Ar-H, pyrazole-5H), 8.56 (s, 1H, pyridine-NH), 12.63 (s, 1H, pyrazole-NH);  $^{13}\text{C NMR}$ :  $\delta$  166.78, 145.23, 136.58, 132.91, 101.39, 52.24, 28.37, 18.22; MS:  $m/z = 437.0 (M + 1)$ ; Anal. calcd. for  $C_{20}H_{19}Cl_2N_3O_4$ : C, 55.06; H, 4.39; N, 9.63; Found: C, 55.03; H, 4.37; N, 9.60%.

#### 4.5.2. Dimethyl 4-[3-(4-(methylsulfanyl)phenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5b**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3252 (N-H-str), 3078, 2946 (C-H-str), 1676 (C=O), 1618 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  2.12 (s, 6H,  $\text{CH}_3$ ), 2.49 (s, 3H,  $\text{SCH}_3$ ), 3.72 (m, 6H, O- $\text{CH}_3$ ), 4.92 (s, 1H, pyridine-4H), 7.25–7.71 (m, 5H, Ar-H, pyrazole-5H), 8.52 (s, 1H, pyridine-

NH), 12.49 (s, 1H, pyrazole-NH); MS:  $m/z = 414.4 (M + 1)$ ; Anal. calcd. for  $C_{21}H_{23}N_3O_4S$ : C, 61.00; H, 5.61; N, 10.16; Found: C, 60.98; H, 5.58; N, 10.14%.

#### 4.5.3. Dimethyl-4-[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5c**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3269 (N-H-str), 3064, 3006 (C-H-str), 1682 (C=O), 1648 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  2.17 (s, 6H,  $\text{CH}_3$ ), 3.32 (s, 6H, O- $\text{CH}_3$ ), 4.79 (s, 1H, pyridine-4H), 7.19–7.36 (m, 2H, 2,5-dichlorothiophene, pyrazole-5H), 8.73 (s, 1H, pyridine-NH), 12.73 (s, 1H, pyrazole-NH); MS:  $m/z = 443.3 (M + 1)$ ; Anal. calcd. for  $C_{18}H_{17}Cl_2N_3O_4S$ : C, 48.88; H, 3.87; N, 9.50; Found: C, 48.85; H, 3.84; N, 9.48%.

#### 4.5.4. Dimethyl-4-[3-(biphenyl-4-yl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5d**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3230 (N-H-str), 3037, 2956 (C-H-str), 1667 (C=O), 1609 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  2.21 (s, 6H,  $\text{CH}_3$ ), 3.19 (s, 6H, O- $\text{CH}_3$ ), 5.09 (s, 1H, pyridine-4H), 7.26 (s, 1H, pyrazole-5H), 7.34–7.81 (m, 9H, Ar-H), 8.88 (s, 1H, pyridine-NH), 12.71 (s, 1H, pyrazole-NH);  $^{13}\text{C NMR}$ :  $\delta$  167.86, 145.58, 140.29, 139.29, 129.45, 128.73, 127.91, 127.01, 126.81, 126.07, 102.71, 50.56, 28.74, 18.45; MS:  $m/z = 444.4 (M + 1)$ ; Anal. calcd. for  $C_{26}H_{25}N_3O_4$ : C, 70.41; H, 5.68; N, 9.47; Found: C, 70.40; H, 5.66; N, 9.45%.

#### 4.5.5. Dimethyl-4-[3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5e**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3202 (N-H-str), 3085, 2952 (C-H-str), 1687 (C=O), 1649 (C=N);  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  2.14 (s, 6H,  $\text{CH}_3$ ), 3.74 (s, 3H, O- $\text{CH}_3$ ), 3.87 (m, 6H, COO- $\text{CH}_3$ ), 5.07 (s, 1H, pyridine-4H), 7.02–7.68 (m, 5H, Ar-H, pyrazole-5H), 8.66 (s, 1H, pyridine-NH), 12.53 (s, 1H, pyrazole-NH); MS:  $m/z = 398.4 (M + 1)$ ; Anal. calcd. for  $C_{21}H_{23}N_3O_5$ : C, 63.46; H, 5.83; N, 10.57; Found: C, 63.44; H, 5.81; N, 10.55%.

#### 4.5.6. Dimethyl 4-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5f**)

IR (KBr,  $\nu_{\max} \text{ cm}^{-1}$ ): 3235 (N-H-str), 3042, 2936 (C-H-str), 1664 (C=O), 1622 (C=N);  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  2.22 (s, 6H,  $\text{CH}_3$ ), 3.18 (s, 6H,  $\text{CH}_2$ ), 4.99 (s, 1H, pyridine-4H), 7.33 (s, 1H, pyrazole-5H), 7.51 (m, 2H, Ar-H), 7.74 (m, 2H, Ar-H), 8.88 (s, 1H, pyridine-NH), 12.64 (s, 1H, pyrazole-NH); MS:  $m/z = 402.8 (M + 1)$ ; Anal. calcd. for  $C_{20}H_{20}ClN_3O_4$ : C, 59.78; H, 5.02; N, 10.46; Found: C, 59.75; H, 4.99; N, 10.43%.

#### 4.6. Antibacterial studies

The antibacterial activity of newly synthesized compounds **4a–f** and **5a–f** were determined by well plate method in Mueller-Hinton Agar. The *in vitro* antibacterial activity was carried out against 24 h old cultures of bacterial strains. In this work, *E. coli*, *S. aureus* and *P. aeruginosa* were used to investigate the activity. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 1 and 0.5 mg/mL. Twenty milliliters of sterilized agar media was poured into each pre-sterilized Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for an hour. About 60  $\mu\text{L}$  of 24 h old culture suspension were poured and neatly swabbed with the pre-sterilized cotton swabs. Six millimeter diameter well were then punched carefully using a sterile cork borer and 30  $\mu\text{L}$  of test solutions of different concentrations were added into each labeled well. The plates were incubated for 24 h at 37 °C. The inhibition zone that appeared after 24 h, around the well in each plate were measured as zone of inhibition in mm. Experiments were in triplicates and standard deviation was calculated.

#### 4.7. Antifungal studies

Antifungal studies of newly synthesized compounds **4a–f** and **5a–f** were carried out against *Aspergillus flavus*, *Chrysosporium Keratinophilum* and *Candida albicans*. Sabourands agar media was prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in distilled water (1000 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37 °C for 1 h. Using sterile cork borer punched carefully, wells were made on these seeded agar plates different concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 25 °C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with fluconazole as standard. Zones of inhibition were determined for compounds **4a–f** and **5a–f**.

#### 4.8. Antioxidant activities

Free radical scavenging activity of the test compounds (**4a–f** and **5a–f**) were carried based on the scavenging activity of stable DPPH. 100 µg/mL of each test sample and standard BHT was taken in different test tubes and the volume was adjusted to 1 mL using MeOH. Freshly prepared 3 mL of 0.1 mM DPPH solution was mixed and vortexed thoroughly and left in dark for 30 min. The absorbance of stable DPPH<sup>•</sup> was measured at 517 nm. The DPPH control (containing no sample) was prepared using the same procedure. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation of DPPH radical scavenging activity.

DPPH radical scavenging activity (%)

$$= \frac{[(\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control}] \times 100}{}$$

where Abs Control is the absorbance of DPPH radical + methanol; Abs Sample is the absorbance of DPPH radical + test sample/standard BHT.

#### 4.9. Acute toxicity and behavioral studies

The acute oral toxicity study for the test compounds (compounds **4c**, **4e** and **4f**) was carried out by following the OECD guidelines. Swiss albino female mice weighing 25–30 g were used for the evaluation. Each group consisting of 6 female mice (overnight fasted) was kept in the colony cage at 25 ± 2 °C with 55% relative humidity and 12 h light/dark cycle was maintained. A Different dose from 250 to 3000 mg/kg was selected and administered orally as a single dose as fine suspension prepared in double distilled water using Tween 80. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4th h and at 8th h, 12th h and 24th h

onset of toxic symptoms and behavioral changes were also recorded.

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