CHAPTER 5

Synthesis, characterization and antimicrobial activity of hydrazone derivatives bearing 2,5-difluoro phenyl ring
CHAPTER-5

Chapter-5: Synthesis, characterization and antimicrobial activity of hydrazone derivatives bearing 2,5-difluoro phenyl ring

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5.1 Introduction

Among the group of biologically active drug molecules, hydrazone derivatives have occupied a significant place in the medicinal chemistry and have fascinated medicinal chemists for their extensive research area. Therapeutic prominence of the hydrazide-hydrazone derivatives has been well documented and were reported to bring forward anti-cancer\textsuperscript{113-121}, anti-HIV properties\textsuperscript{122} and hence they have gained a crucial place in medicinal chemistry. Hydrazide-hyrazones are well thought-out to be good scaffolds for various pharmaceutical applications and were evaluated for various potent biological studies \textit{viz.}, anti-microbial, anti-fungal, anti-bacterial, and anti-convulsant agents\textsuperscript{123-128}. Because of the diverse biological properties of hydrazide-hydrazone derivatives, in recent years they have gained great importance that includes anti-tuberculotic, anti-inflammatory, anti-malarial activities\textsuperscript{129-131}.

Even though there exists a large number of antibiotics and chemotherapeutics accessible for medical use, the anti-microbial resistance has formed a considerable need for design of innovative class of antimicrobials and this area of subject will always remain a vicinity of enormous significance.

In the previous chapter (Chapter 3) we found that, 2,5-difluoro-benzohydrazides when condensed with different aromatic aldehydes has shown potential anti-bacterial activity. As a continuation, the aryl group which contains the difluoro was extended with another aryl group substituted with chlorine atom and the obtained hydrazide was
then condensed with various aldehydes to prepare novel library of hydrazone derivatives.

5.2 Objective of the work

The present chapter deals with the synthesis of 4-(2,5 difluorobenzyloxy)-3-chlorobenzohydrazide and reacting it with appropriate aromatic aldehydes to get novel hydrazone derivatives. The identification of the prepared derivatives was done by the NMR, MS, and IR spectroscopy. The obtained derivatives screened for the microbial studies to access their biological activity.

Scheme 5.1: Retro synthesis aspect of 2,5-difluoro phenyl hydrazone derivatives

5.3 Results and discussion:

5.3.1 Synthesis

The reaction progression for the synthesis of fifteen new hydrazone derivatives 9a-9o is presented in Scheme 1. 2,5-difluoro benzoic 1 was converted to the corresponding methyl benzoate
derivative 2 in presence of H$_2$SO$_4$ (catalytic quantity) in methanol. The methyl benzoate derivative 2 was then treated with sodium borohydride in methanol to afford the benzyl alcohol derivative 3. Benzyl alcohol derivative 3 was converted to benzyl bromide derivative 4 using 33% HBr in acetic acid. Esterification of 3-chloro-4-hydroxybenzoic acid 5 was carried out using conc. H$_2$SO$_4$ in methanol to afford methyl benzoate derivative 6. Coupling of benzyl bromide derivative 4 with methyl benzoate derivative 6 using potassium carbonate in DMF gave compound 7. Compound 7 was then treated with hydrazine hydrate in ethanol to afford the key intermediate hydrazide derivative 8. Condensation reaction between hydrazide 8 and various benzaldehydes, lead to hydrazide-hydrazone derivatives 9a-9o. The synthesized hydrazide-hydrazone derivatives 9a-9o was characterized by IR, $^1$H NMR and $^{13}$C NMR, mass and other spectral data.

5.3.2 Spectral interpretation (Compound 9l)

![Fig. 5.1](image)

The $^1$H NMR spectrum of compound 9l showed two singlet’s at $\delta$ 11.82 ppm and 8.42 ppm corresponding to the protons attached to -CONH and -CH=N functional group respectively. A singlet proton at
8.04 ppm corresponds to the H₃ proton flanked adjacent to the chloride group of ring B, while the H₃’ proton with a doublet signal resonates at 7.92 pp (J = 6.2 Hz). A doublet (d) was located at δ 7.80 (J = 4.8 Hz), ppm which indicated two aromatic H₁ & H₁’ and another doublet (d) at δ 7.42 (J = 6.8 Hz) ppm was assigned for two aromatic H₂ & H₂’ protons of the C ring. A multiplet resonating between δ 7.40 - 7.24 ppm was assigned to the three aromatic H-3, H-5, H-5’ and H-5” protons of A and B rings respectively. Finally, the methylene (-OCH₂) protons attached to the oxygen atom (ether linkage between ring A and B) resonates at 5.30 ppm as singlet.

The IR data suggest an absorption band at 1598 cm⁻¹ is due to aromatic (C=C) stretching. Another absorption band at 1057 cm⁻¹ is due to (C-N) stretching. The presence of a carbonyl group (C=O) was assigned on the basis of an absorption band at 1649 cm⁻¹ and the absorption band at 3220 cm⁻¹ is due to presence of NH stretching.

Furthermore, the fact was also supported by the mass spectrum of compound 91 which showed a molecular ion peak (M+) at m/z 419.10 (M+1). These data are satisfactory for the structure assigned to the compound.
5.3.3 Reaction scheme

Scheme 5.2: Synthesis of hydrazine derivatives 9a – 90

Table 5.1: Molecules prepared from 2, 5-difluorobenzoic acid

<table>
<thead>
<tr>
<th>R</th>
<th>Structure</th>
<th>Compound</th>
<th>Formula</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-di-OCH₃</td>
<td><img src="image" alt="Structure" /></td>
<td>9a</td>
<td>C₂₃H₁₉ClF₂N₂O₄</td>
<td>85%</td>
</tr>
<tr>
<td>R</td>
<td>Structure</td>
<td>Compound</td>
<td>Formula</td>
<td>Yield %</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------</td>
<td>----------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>2,5-di-OCH₃</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>9b</td>
<td>C₂₃H₁₉ClF₂N₂O₄</td>
<td>90%</td>
</tr>
<tr>
<td>2,6-di-OCH₃</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>9c</td>
<td>C₂₃H₁₉ClF₂N₂O₄</td>
<td>93%</td>
</tr>
<tr>
<td>3,4,5 tri-OCH₃</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>9d</td>
<td>C₂₄H₂₁ClF₂N₂O₅</td>
<td>96%</td>
</tr>
<tr>
<td>3- OCH₃-4- OC₂H₅</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>9e</td>
<td>C₂₄H₂₁ClF₂N₂O₄</td>
<td>97%</td>
</tr>
<tr>
<td>3- OCH₃-4- OC₃H₇</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>9f</td>
<td>C₂₅H₂₃ClF₂N₂O₄</td>
<td>94%</td>
</tr>
<tr>
<td>R</td>
<td>Structure</td>
<td>Compound</td>
<td>Formula</td>
<td>Yield %</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>----------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>4-t-butyl</td>
<td><img src="image1" alt="Structure" /></td>
<td>9g</td>
<td>C_{25}H_{23}ClF_{2}N_{2}O_{2}</td>
<td>92%</td>
</tr>
<tr>
<td>4-CF₃</td>
<td><img src="image2" alt="Structure" /></td>
<td>9h</td>
<td>C_{22}H_{14}ClF_{3}N_{2}O_{2}</td>
<td>95%</td>
</tr>
<tr>
<td>4-OCF₃</td>
<td><img src="image3" alt="Structure" /></td>
<td>9i</td>
<td>C_{22}H_{14}ClF_{3}N_{2}O_{3}</td>
<td>89%</td>
</tr>
<tr>
<td>3-CF₃</td>
<td><img src="image4" alt="Structure" /></td>
<td>9j</td>
<td>C_{22}H_{14}ClF_{3}N_{2}O_{2}</td>
<td>92%</td>
</tr>
<tr>
<td>2-CF₃</td>
<td><img src="image5" alt="Structure" /></td>
<td>9k</td>
<td>C_{22}H_{14}ClF_{3}N_{2}O_{2}</td>
<td>88%</td>
</tr>
<tr>
<td>R</td>
<td>Structure</td>
<td>Compound</td>
<td>Formula</td>
<td>Yield %</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------</td>
<td>----------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>4-F</td>
<td><img src="image" alt="Structure" /></td>
<td>9l</td>
<td>C_{21}H_{14}Cl F_{3}N_{2}O_{2}</td>
<td>91%</td>
</tr>
<tr>
<td>2,4-di-F</td>
<td><img src="image" alt="Structure" /></td>
<td>9m</td>
<td>C_{21}H_{13}Cl F_{4}N_{2}O_{2}</td>
<td>93%</td>
</tr>
<tr>
<td>4-Br</td>
<td><img src="image" alt="Structure" /></td>
<td>9n</td>
<td>C_{21}H_{14}BrCl F_{2}N_{2}O_{2}</td>
<td>90%</td>
</tr>
<tr>
<td>Benzo furan</td>
<td><img src="image" alt="Structure" /></td>
<td>9o</td>
<td>C_{29}H_{19}Cl F_{3}N_{2}O_{3}</td>
<td>96%</td>
</tr>
</tbody>
</table>

### 5.3.4 Antimicrobial evaluation

The outcome of anti-bacterial screening of fifteen newly synthesized entities 9a-9o are accessible in Table 1. Results discloses that four chosen bacterial strains like, E. coli, P. aeruginosa, S. aureus and S. pyogenes has revealed different patterns of activities against the control drug ampicillin. For, E. coli, compound 9h (R = 4-CF₃), 9k (R = 2- CF₃) and 9m (R = 2,4-difluoro) displayed excellent activity
While compounds $9d$ ($R = 3,4,5$-$\text{OMe}$), $9i$ ($R = 4$-$\text{OCF}_3$), and $9o$ ($R = \text{Benzo}[b]\text{furan}$) showed equipotent activity (zone of inhibition: 19-22 mm) and the compounds $9e$, $9f$, $9j$ and $9l$ ($R = 3$-$\text{OMe}$, 4-$\text{OEt}$, 3-$\text{OMe}$, 4-$\text{OPr}$, 3-$\text{CF}_3$, 4-$\text{F}$ respectively) exhibited good activity (zone of inhibition: 17–20 mm) and the remaining entities in the series such as $9a$ ($R = 2$,$4$-$\text{OMe}$), $9b$ ($R = 2$,$5$-$\text{OMe}$) and $9c$ ($R = 2$,$6$-$\text{OMe}$) showed moderate activity (zone of inhibition: 13-16 mm) when compared to the standard drug ampicillin. Similar trends of antibacterial activity were observed when tested against the following bacterial strains such as $\text{Pseudomonas aeruginosa}$ (MTCC 424), $\text{Staphylococcus aureus}$ (MTCC 96), and $\text{Staphylococcus pyogenes}$ (MTCC 442). Compounds $9g$ ($R = t$-$\text{butyl}$) and $9n$ ($R = \text{Br}$) was found to be inactive against all the tested bacterial strains. As most of the tested entities emerged as active against all the tested microorganisms, it indicates that this essential scaffold can be a promising anti-bacterial drug agent. Hence hydrazones derivative with an appropriate $R$ group may emerge as a good anti-bacterial agent for all the $\text{Escherichia coli}$, $\text{Pseudomonas aeruginosa}$, $\text{Streptococcus pyogenes}$ and $\text{Staphylococcus aureus}$ bacterial strains.

5.4 Anti-bacterial evaluation procedure and results

The prepared new derivatives ($9a$-$9o$) were tested for the anti-bacterial activity against the two gram-negative and two gram-positive bacteria by agar well diffusion method. $\text{Escherichia coli}$ (MTCC 443), $\text{Pseudomonas aeruginosa}$ (MTCC 424) are the two gram positive
organisms used and *Staphylococcus aureus* (MTCC 96), and *Staphylococcus pyogenes* (MTCC 442) are the two gram negative organisms used for the testing. The procedure for the conducting the antibacterial activity was followed as per the anti-bacterial evaluation procedure given in the section 4.4 of the chapter-4.

The results of the bacterial study is tabulated below.

**Table 5.2: Results of antibacterial bioassay of compounds 9a -9o**

(Concentration Used 250 µg mL⁻¹ of DMSO).

<table>
<thead>
<tr>
<th>Cmp No.</th>
<th>R</th>
<th>Gram-negative</th>
<th>Gram-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.Coli</td>
<td>P.aeruginosa</td>
</tr>
<tr>
<td>9a</td>
<td>2,4-OMe</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>9b</td>
<td>2,5-di-OMe</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>9c</td>
<td>2,6-OMe</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>9d</td>
<td>3,4,5-OMe</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>9e</td>
<td>3-OCH₃, 4-OC₂H₅</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>9f</td>
<td>3-OCH₃, 4-OC₃H₇</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>9g</td>
<td>4-t-butyl</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9h</td>
<td>4-CF₃</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>9i</td>
<td>4-OCF₃</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>9j</td>
<td>3-CF₃</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>9k</td>
<td>2-CF₃</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>9l</td>
<td>4-F</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>9m</td>
<td>2,4-di-F</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>9n</td>
<td>4-Br</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Cmp No.</td>
<td>R</td>
<td>Gram-negative</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E.Coli</td>
<td>P.aeruginosa</td>
</tr>
<tr>
<td>9o</td>
<td>Benzo[b]furan</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Standard Drug</td>
<td>Ampicillin (250 µg/mL of DMSO)</td>
<td>22</td>
<td>20</td>
</tr>
</tbody>
</table>

Zones of Inhibition of compounds 9a – 9o in mm

The results are plotted on the graph shown below.

Fig. 5.2: Graphical representation of anti-bacterial bioassay of compounds 9a-9o.
5.5 Experimental procedures and characterization data.

5.5.1 Experimental procedures and spectral data for compounds 2 to 8

Synthesis of methyl 2,5-difluorobenzoate (2):

To a solution of compound 1 (3 g, 18.98 mmol) in methanol (30 mL) was added sulphuric acid (0.5 mL) and refluxed for 25 h. After completion of reaction, methanol was evaporated under reduced pressure and the obtained residue was taken in ethylacetate (75 mL) and washed with 10% aq. NaHCO₃ solution (2 x 10 mL) followed by water and brine solution. The organic layer was separated, dried over Na₂SO₄, filtered and evaporated to afford compound 2.

Colorless liquid, yield: 2.5 g, 76%; b.p: 211-212°C; IR (KBr): \( \nu_{\text{max}} \) 3082, 3005, 2957, 1883, 1739, 1625, 1597, 1496, 1142, 1420, 1312, 1274, 1251, 1188, 1122, 1075, 985, 891, 827, 805, 782, 689, 671 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl₃): \( \delta \) 7.68-7.64 (m, 1 H), 7.62-7.54 (m, 1 H), 7.48-7.46 (m, 1 H), 3.84 (s, 3 H); ESI-MS: \( m/z \) (rel.abund.%): 173.10 (M+, 100); ESI-HRMS \( m/z \): calcd for C₈H₇N₂O₂F₂ ([M+H]+): 173.0414; found: 173.0419.

Synthesis of (2,5-difluorophenyl)methanol (3)

To a solution of compound 2 (6 g, 34.85 mmol) in methanol (30 mL), cooled to 5-10°C was added sodium borohydride (3 g, 37.84 mmol) in six portions. The reaction mixture was allowed to stir at room temperature for 3.5 h. The reaction mixture was quenched with water (2 mL) and evaporated under pressure to obtain a yellow residue. The residue was taken in ethyl acetate (100 mL) and washed with water
followed by brine solution. The organic layer was separated, dried over Na2SO4, filtered and evaporated to compound 3.

Colorless liquid, yield: 4.5 g, 90%; b.p: 196 – 197°C; 1H NMR (400 MHz, CDCl3): δ 7.60-7.21 (m, 3 H), 5.40 (t, 1 H, J = 5.8 Hz), 4.58 (d, 2 H, J = 4.8 Hz);

**Synthesis of 2-(bromomethyl)-1, 4-difluorobenzene (4)**

A mixture of compound 3 (5 g, 34.70 mmol) in 33% HBr in acetic acid (25 mL) was heated to 55°C for 2.5 h. The reaction mixture was diluted with water (25 mL) and extracted with dichloro methane (2 x 20 mL). The organic layer washed with 10% aq. NaHCO3 solution (3 x 15 mL) followed by water and brine solution. The organic layer was separated, dried over Na2SO4, filtered and evaporated to afford compound 4.

Reddish brown liquid, yield: 5 g, 69%; b.p: 186 – 189°C; 1H NMR (400 MHz, CDCl3): δ 7.48-7.42 (m, 1 H), 7.34-7.22 (m, 2H), 4.62 (s, 2 H);

**Synthesis of methyl 3-chloro-4-hydroxybenzoate (6)**

To a solution of compound 5 (5 g, 29.06 mmol) in methanol (50 mL) was added sulphuric acid (0.1 mL) and refluxed for 6 h. After completion of the reaction, methanol was evaporated under reduced pressure and the obtained residue was taken in ethylacetate (60 mL), washed with 10% aq. NaHCO3 solution (3 x 10 mL) followed by water and brine solution. The organic layer was separated, dried over Na2SO4, filtered and evaporated to afford compound 6.

White solid, yield: 4.0 g, 73%; m.p: 108 – 110°C; IR (KBr): νmax 3346, 2957, 1690, 1604, 1577, 1512, 1443, 1418, 1355, 1292, 1268, 1189,
1126, 1054, 972, 909, 877, 830, 806, 765, 711, 656, 633, 544; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 11.30 (br.s, 1 H), 7.87 (d, 1 H, \textit{J} = 2.4 Hz), 7.67 (dd, 1 H, \textit{J} = 2.0, 2.4 Hz), 7.05 (d, 1 H, \textit{J} = 12.0 Hz), 3.82 (s, 3 H);

**Synthesis of methyl 4-(2,5-difluorobenzyloxy)-3-chlorobenzoate (7)**

To a stirred mixture of compound 6 (2.4 g, 12.85 mol) and potassium carbonate (1.77 g, 12.85 mol) in dimethyl formamide (15 mL), at room temperature was added compound 4 (2 g, 9.66 mmol) over a period of 5 min. The reaction mixture was heated to 70°C for 2 h. The reaction mixture was diluted with water (25 L) and extracted with ethyl acetate (30 mL). Organic layer was separated and washed with water (3 x 25 mL) followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated in \textit{vacuo} to give crude compound, which was purified by flash chromatography eluting with hexane-ethyl acetate (90:10) to afford compound 8 as a white solid.

White solid, yield: 2 g, 60%; m.p: 124 – 125°C; IR (KBr): \textit{\nu} \text{max} 3087, 3030, 2956, 1715, 1504, 1489, 1458, 1435, 1408, 1383, 1286, 1294, 1235, 1191, 1183, 1122, 1091, 1063, 1033, 978, 957, 905, 877, 817, 788, 763, 729, 647, 568; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 7.98-7.92 (m, 2H), 7.48-7.42 (m, 2 H), 7.40-7.28 (m, 2 H), 5.40 (s, 2 H), 3.86 (s, 3 H); ESI-MS: m/z, 313.10 (M+1); ESI-HRMS m/z: calcd for C\textsubscript{15}H\textsubscript{12}O\textsubscript{3}F\textsubscript{2}Cl ([M+H]\textsuperscript{+}): 313.0443; found: 313.0445.
Synthesis of 4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide(8)

To a solution of compound 7 (1.5 g, 4.80 mmol) in ethanol (15.0 mL) was added hydrazine hydrate (14.40 mmol) and heated to reflux for 10 h. After completion of the reaction, ethanol was concentrated under reduced pressure to obtain crude compound 8. The crude compound was slurred in n-Hexane, filtered at the high vacuum pump and dried to obtain compound 8. White solid, yield: 1.3 g, 86%; m.p: 87 – 88°C; $^1$H NMR (400 MHz, CDCl3): $\delta$ 9.80 (s, 1 H), 7.94 (s, 1 H), 7.84 (d, 1 H, $J = 4.2$ Hz), 7.50-7.18 (m, 4 H), 5.25 (s, 2 H), 4.50 (s, 2 H).
5.5.2 General experimental procedure for the synthesis of hydrazone derivatives (9a-9o):

To a stirred solution of compound 8 (100 mg, 0.32 mmol) in ethanol was added corresponding benzaldehydes (1.0 mmol) and refluxed for 3 h. The reaction conversion was monitored by TLC. After completion of the reaction, the reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain the pure compounds. Yields of the products varied between 85 and 97%.

5.5.3 Physical properties and Spectral data for hydrazone derivatives (9a-9o):

(E)-N’-(2,4-dimethoxybenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9a):

White solid; m.p: 117-118°C; IR (KBr): \( \nu_{\max} \) 3179, 3043, 2978, 2838, 1658, 1636, 1602, 1558, 1434, 1456, 1383, 1275, 1209, 1189, 1156, 1107, 1064, 1034, 937, 926, 833, 756, 730. cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 11.60 (s, 1 H), 8.78 (s, 1 H), 8.06 (s, 1 H), 7.83 (dd, 1 H, \( J = 1.6, 8.8 \) Hz), 7.80 (d, 1 H, \( J = 9.2 \) Hz), 7.48 - 7.26 (m, 4 H), 6.65 (s,
2 H), 3.80 (s, 3 H), 3.84 (s, 3 H), 5.30 (s, 2 H); $^{13}$C NMR (CDCl$_3$): δ 162.44, 160.79, 159.25, 159.13, 157.52, 156.86, 155.64, 155.09, 143.26, 129.17, 128.21, 127.0, 126.63, 121.39, 115.07, 113.73, 64.26, 55.73, 55.14; ESI-MS: m/z, 461.10 (M+1); ESI-HRMS m/z: calcd for C$_{23}$H$_{20}$N$_2$O$_4$F$_2$Cl ([M+H]$^+$): 461.1080; found: 461.1060.

**{(E)}-N’-(2,5-dimethoxybenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9b):**

![Chemical Structure](image)

White solid; m.p: 107-108°C; IR (KBr): $\nu_{\text{max}}$ 3181, 3078, 2991, 2835, 1644, 1609, 1561, 1493, 1463, 1431, 1358, 1276, 1243, 1222, 1189, 1171, 1110, 1045, 961, 925, 878, 812, 763, 756, 708, 690, 651, 610, 595, 437 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): δ 11.98 (s, 1 H), 8.80 (s, 1 H), 8.08 (dd, 1 H, $J$ = 2.0 Hz), 7.96 (dd, 1 H, $J$ = 1.6, 8.0 Hz), 7.48-7.26 (m, 5 H), 7.08 – 7.02 (m, 2 H), 5.30 (s, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H); $^{13}$C NMR (CDCl$_3$): δ 161.02, 159.23, 157.49, 156.86, 155.77, 155.08, 153.23 (2C), 152.28 (2C), 143.05 (2C), 129.24, 128.31, 126.79, 122.84, 121.43, 113.76, 113.40, 109.21, 64.28, 56.20, 55.43; ESI-MS: m/z, 461.10 (M+1); ESI-HRMS m/z: calcd for C$_{23}$H$_{20}$N$_2$O$_4$F$_2$Cl ([M+H]$^+$): 461.1080; found: 461.1086.
(E)-N'-(2,6-dimethoxybenzylidene)-4-(2,5-difluorobenzylxyloxy)-3-chlorobenzohydrazide (9c):

White solid; m.p: 127-128°C; IR (KBr): \( \nu_{\text{max}} \) 3192, 3012, 2938, 2837, 1645, 1597, 1556, 1497, 1469, 1431, 1379, 1312, 1257, 1189, 1142, 1115, 1064, 1034, 956, 942, 910, 877, 810, 778, 755, 731, 649, 477 cm\(^{-1}\); 1H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 11.60 (s, 1 H), 8.60 (s, 1 H), 8.08 (s, 1 H), 7.92 (d, 1 H, \( J = 5.4 \) Hz), 7.50-7.22 (m, 5 H), 6.72 (d, 2 H, \( J = 5.8 \) Hz), 5.20 (s, 2 H), 3.80 (s, 6 H); ESI-MS: m/z, 461.10 (M+1); ESI-HRMS m/z: calcd for C\(_{23}\)H\(_{20}\)N\(_2\)O\(_4\)F\(_2\)Cl ([M+H]\(^+\)): 461.1080; found: 461.1082.

(E)-4-(2,5-difluorobenzylxyloxy)-N'-(3,4,5-trimethoxybenzylidene)-3-chlorobenzohydrazide(9d):

White solid; m.p: 122-124°C; IR (KBr): \( \nu_{\text{max}} \) 3439, 3207, 3063, 2948, 2839, 1649, 1600, 1501, 1468, 1364, 1297, 1272, 1188, 1128, 1068,
1056, 997, 947, 900, 881, 841, 828, 818, 788, 743, 722, 751, 703, 669, 644, 605, 518, 492 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 11.90 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 7.90 (d, 1 H, \(J = 7.2\) Hz), 7.50-7.24 (m, 4 H), 7.0 (s, 1 H), 5.20 (s, 2 H), 3.84 (s, 6 H), 3.82 (s, 3 H); \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 161.21, 159.24, 157.05, 156.84, 155.75, 155.01, 153.18 (3C), 147.79 (2C), 139.24, 129.77, 129.25, 128.30, 126.93, 125.17, 121.46, 113.80, 104.28, 64.28, 60.10, 55.93 (2C); ESI-MS: m/z, 491.10 (M+1); ESI-HRMS m/z: calcd for C\(_{24}\)H\(_{22}\)N\(_2\)O\(_5\)F\(_2\)Cl ([M+H]\(^+\)): 461.1185; found: 491.1180.

\((E)\)-4-(2,5-difluorobenzyloxy)-N'-\((4\text{-ethoxy-3-methoxybenzylidene})-3\text{-chlorobenzohydrazide(9e):}\)

![Structure diagram](image)

White solid; m.p: 97-98°C; IR (KBr): \(\nu_{\text{max}}\) 3217, 3078, 2974, 2935, 1642, 1599, 1573, 1544, 1500, 1470, 1456, 1420, 1385, 1369, 1334, 1297, 1272, 1214, 1191, 1174, 1140, 1107, 1064, 1035, 969, 961, 932, 906, 858, 873, 805, 754, 766, 731, 696, 651, 621, 514, 488, 448 cm\(^{-1}\); 1H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 11.70 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 7.94 (d, 1 H, \(J = 8.4\) Hz), 7.58-7.22 (m, 5 H), 7.20 (d, 1 H, \(J = 8.8\) Hz), 5.38 (s, 2 H), 4.08 (q, 2 H, \(J = 6.8\) Hz), 3.80 (s, 3 H), 1.40 (t, 3 H, \(J = 6.6\) Hz); ESI-MS: m/z, 475.20 (M+1).
(E)-4-(2,5-difluorobenzyloxy)-N'-(3-methoxy-4 propoxybenzylidene) -3-chlorobenzohydrazide (9f):

White solid; m.p: 132-133°C; IR (KBr): $\nu_{\text{max}}$ 3226, 3074, 2937, 1644, 1599, 1574, 1545, 1502, 1469, 1456, 1421, 1384, 1372, 1332, 1297, 1273, 1239, 1191, 1174, 1140, 1064, 1034, 969, 904, 873, 862, 846, 797, 731, 685, 873, 862, 846, 797, 731, 685, 651, 596, 566, 518, 491, 448 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 11.70 (s, 1 H), 8.40 (s, 1 H), 8.06 (s, 1 H), 7.92 (d, 1 H, $J = 9.2$ Hz), 7.54-7.24 (m, 5 H), 7.20 (d, 1 H, $J = 8.0$ Hz), 7.0 (d, 1 H, $J = 8.2$ Hz), 5.38 (s, 2 H), 4.02 (q, 2 H, $J = 6.8$ Hz), 1.70 (q, 2 H, $J = 6.8$ Hz), 1.0 (t, 3 H, $J = 7.2$ Hz); $^{13}$C NMR (CDCl$_3$): δ 161.05, 159.24, 157.82, 156.86, 155.69, 150.18, 149.19, 148.01, 129.20, 128.24, 127.04, 126.86, 125.01, 121.91, 121.44, 113.79 (2C), 112.42 (2C), 108.44 (2C), 69.63, 55.48, 22.0, 10.39; ESI-MS: m/z, 489.20 (M+1).
(E)-4-(2,5-difluorobenzyloxy)-N’-(4-tert-butylbenzylidene)-3-chlorobenzohydrazide (9g):

White solid; m.p: 92-94°C; IR (KBr): $\nu_{\text{max}}$ 3358, 3079, 2963, 1667, 1609, 1598, 1565, 1532, 1495, 1459, 1432, 1388, 1371, 1306, 1262, 1189, 1178, 1147, 1134, 1064, 1029, 976, 961, 939, 881, 836, 825, 805, 751, 734, 723, 708, 647, 593, 510, 448 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 11.80 (s, 1 H), 8.42 (s, 1 H), 8.04 (s, 1 H), 7.92 (d, 1 H, $J$ = 9.6 Hz), 7.66 (d, 2 H, $J$ = 8.0 Hz), 7.52-7.26 (m, 6 H), 5.38 (s, 2 H), 1.0 (s, 9 H); $^{13}$C NMR (CDCl$_3$): δ 161.16, 159.24, 157.50, 156.87, 155.76, 155.09, 152.90, 147.76, 131.55, 129.25, 128.29, 126.92 (2C), 125.63 (2C), 125.18, 121.46, 113.78 (2C), 64.30, 34.58, 30.93 (4C); ESI-MS: m/z, 457.20 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N’-(4-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9h):

White solid; m.p: 92-94°C; IR (KBr): $\nu_{\text{max}}$ 3358, 3079, 2963, 1667, 1609, 1598, 1565, 1532, 1495, 1459, 1432, 1388, 1371, 1306, 1262, 1189, 1178, 1147, 1134, 1064, 1029, 976, 961, 939, 881, 836, 825, 805, 751, 734, 723, 708, 647, 593, 510, 448 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 11.80 (s, 1 H), 8.42 (s, 1 H), 8.04 (s, 1 H), 7.92 (d, 1 H, $J$ = 9.6 Hz), 7.66 (d, 2 H, $J$ = 8.0 Hz), 7.52-7.26 (m, 6 H), 5.38 (s, 2 H), 1.0 (s, 9 H); $^{13}$C NMR (CDCl$_3$): δ 161.16, 159.24, 157.50, 156.87, 155.76, 155.09, 152.90, 147.76, 131.55, 129.25, 128.29, 126.92 (2C), 125.63 (2C), 125.18, 121.46, 113.78 (2C), 64.30, 34.58, 30.93 (4C); ESI-MS: m/z, 457.20 (M+1).
White solid; m.p: 138-140°C; IR (KBr): $\nu_{\text{max}}$ 3219, 3062, 1649, 1597, 1544, 1502, 1434, 1415, 1403, 1378, 1360, 1333, 1293, 1275, 1233, 1192, 1152, 1114, 1070, 1017, 996, 961, 943, 905, 881, 842, 833, 785, 751, 720, 678, 640, 599, 504, 474, 447 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.0 (s, 1 H), 8.60 (s, 1 H), 8.07 (s, 1 H), 7.98-7.61 (m, 3 H), 7.81 (d, 2 H, $J = 8.4$ Hz), 7.49-7.26 (m, 4 H), 5.40 (s, 2 H); $^{13}$C NMR (CDCl$_3$): $\delta$ 161.13, 159.24, 157.43, 156.85, 155.93, 155.08, 138.25, 129.83, 129.51, 129.35, 128.44, 127.63, 126.60, 125.72, 125.68, 125.43, 125.41, 125.07, 122.72, 121.50, 113.80, 64.30; ESI-MS: m/z, 469.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-(trifluoromethoxy)benzylidene)-3-chlorobenzohydrazide (9i):

White solid; m.p: 143-145°C; IR (KBr): $\nu_{\text{max}}$ 3222, 3064, 1647, 1608, 1598, 1544, 1502, 1434, 1403, 1363, 1309, 1290, 1272, 1233, 1193, 1151, 1103, 1057, 1018, 998, 957, 937, 906, 881, 846, 813, 785, 752, 720, 704, 649, 517, 446 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d6): $\delta$ 11.90 (s, 1 H), 8.48 (s, 1 H), 8.06 (s, 1 H), 7.96 (d, 1 H, $J = 6.4$ Hz), 7.84 (d, 2 H, $J = 7.2$ Hz), 7.50-7.24 (m, 6 H), 5.40 (s, 2 H); ESI-MS: m/z, 485.10 (M+1).
(E)-4-(2,5-difluorobenzyloxy)-N’-(3-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9j):

White solid; m.p: 136-138°C; IR (KBr): $\nu_{\text{max}}$ 3188, 2998, 1652, 1603, 1566, 1501, 1493, 1456, 1431, 1369, 1328, 1313, 1273, 1243, 1215, 1191, 1166, 1147, 1120, 1097, 1068, 1030, 954, 910, 879, 862, 823, 806, 757, 721, 713, 697, 670, 626, 495 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d6): $\delta$ 12.0 (s, 1 H), 8.50 (s, 1 H), 8.14-7.92 (m, 4 H), 7.84-7.68 (m, 2 H), 7.56-7.24 (m, 4 H), 5.30 (s, 2 H); $^{13}$C NMR (CDCl$_3$): $\delta$ 167.43, 161.44, 159.25, 157.52, 156.85, 155.91, 155.11, 153.33, 145.93, 135.47, 131.06, 130.04, 129.34, 128.45, 126.62, 125.36, 129.14, 122.95, 121.49, 113.80, 64.32; ESI-MS: m/z, 469.10 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N’-(2-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9k):

White solid; m.p: 103-105°C; IR (KBr): $\nu_{\text{max}}$ 3184, 3014, 1651, 1603, 1563, 1500, 1493, 1462, 1430, 1377, 1312, 1271, 1214, 1192, 1176,
1163, 1120, 1145, 1066, 1032, 942, 911, 886, 863, 822, 810, 766, 770, 756, 712, 682, 642, 625, 595, 571, 448, 409 cm⁻¹; ¹H NMR (400 MHz, DMSO-d6): δ 12.10 (s, 1 H), 8.80 (s, 1 H), 8.26 (d, 1 H, J = 7.2 Hz), 8.08 (s, 1 H), 7.96 (d, 1 H, J = 6.8 Hz), 7.66 (t, 1 H, J = 6.4 Hz), 7.56-7.32 (m, 4 H), 5.30 (s, 2 H); ¹³C NMR (CDCl₃): δ 161.42, 159.24, 157.52, 156.52, 156.85, 155.98 (2C), 155.09, 142.64, 132.82, 132.15, 130.08, 129.31, 128.50, 126.86, 126.45, 125.88, 125.53, 124.88, 121.50, 116.99, 113.83, 64.30; ESI-MS: m/z, 469.00 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-fluorobenzylidene)-3-chlorobenzohydrazide (9l):

White solid; m.p: 112-114°C; IR (KBr): ν_max 3220, 3071, 3039, 1649, 1606, 1598, 1565, 1548, 1511, 1500, 1415, 1433, 1403, 1365, 1296, 1275, 1233, 1217, 1191, 1157, 1143, 1099, 1057, 1015, 998, 958, 933, 904, 879, 832, 815, 790, 752, 721, 664, 644, 522, 462 cm⁻¹; ¹H NMR (400 MHz, DMSO-d6): δ 11.82 (s, 1 H), 8.42 (s, 1 H), 8.04 (s, 1 H), 7.92 (d, 1 H, J = 6.2 Hz), 7.80 (t, 2 H, J = 4.8 Hz), 7.42 (d, 2 H, J = 6.8 Hz), 7.40-7.24 (m, 4 H), 5.30 (s, 2 H); ¹³C NMR (CDCl₃): δ 164.35, 161.88, 161.24 (2C), 159.25, 157.52, 156.85, 155.80 (2C), 155.11, 146.52 (2C), 130.89, 129.27, (2C), 128.34 (2C), 126.81, 121.47, 113.80, 64.29; ESI-MS: m/z, 419.10 (M+1).
(E)-N’-(2,4-difluorobenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9m):

White solid; m.p: 101-102°C; IR (KBr): $\nu_{\text{max}}$ 3228, 3086, 1639, 1618, 1599, 1569, 1565, 1459, 1426, 1408, 1388, 1366, 1301, 1280, 1270, 1243, 1188, 1175, 1143, 1092, 1062, 1032, 967, 871, 915, 850, 814, 756, 732, 682, 651, 613, 509, 487, 445, 487, 421 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d6): $\delta$ 11.95 (s, 1 H), 8.64 (s, 1 H), 8.08 (s, 1 H), 8.04-7.90 (m, 2 H), 7.50-7.18 (m, 6 H), 5.30 (s, 2 H); ESI-MS: m/z, 437.0 (M+1).

(E)-4-(2,5-difluorobenzyloxy)-N’-(4-bromobenzylidene)-3-chlorobenzohydrazide (9n):

White solid; m.p: 87-88°C; IR (KBr): $\nu_{\text{max}}$ 3183, 3064, 1644, 1598, 1566, 1546, 1499, 1431, 1405, 1362, 1295, 1270, 1213, 1190, 1143, 1099, 1055, 1010, 1001, 974, 940, 894, 884, 862, 818, 782, 748, 721, 702, 669, 515, 466, 443, 414 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-
\[ \delta 11.96 \text{ (s, 1 H)}, 8.40 \text{ (s, 1 H)}, 8.06 \text{ (s, 1 H)}, 8.12 \text{ (d, 1 H, J = 7.2 Hz)}, \]
\[ 7.74-7.62 \text{ (m, 4 H)}, 7.38-7.22 \text{ (m, 4 H)}, 5.30 \text{ (s, 2 H)}; \text{ESI-MS: m/z, 479.0 (M+1)}. \]

\textit{(E)-4-(2,5-difluorobenzyloxy)-N'-((benzofuran-2-yl)methylene)-3-chlorobenzohydrazide (9o):}

\[
\begin{align*}
\text{White solid; m.p: 111-112^\circ C;} & \text{ IR (KBr): } \nu_{\text{max}} 3228, 3050, 2553, 1650, 1619, 1598, 1556, 1535, 1496, 1457, 1446, 1382, 1353, 1307, 1266, 1239, 1190, 1148, 1108, 1034, 926, 962, 888, 855, 820, 802, 747, 733, 679, 613, 443 \text{ cm}^{-1}; \quad ^1\text{H NMR (400 MHz, DMSO-d6): } \delta 12.0 \text{ (s, 1 H)}, 8.50 \text{ (s, 1 H)}, 8.10 \text{ (s, 1 H)}, 7.96 \text{ (d, 1 H, J = 6.6 Hz)}, 7.80-7.62 \text{ (m, 2 H)}, 7.52-7.24 \text{ (m, 7 H)}, 5.40 \text{ (s, 2 H)}; \text{ESI-MS: m/z, 441.10 (M+1)}. \end{align*}
\]
5.6 Spectra for selected compounds

4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide(8).

Fig. 5.3: \(^1\)H NMR spectrum of 8

Fig. 5.4: Mass spectrum of 8
(E)-N’-(2,4-dimethoxybenzylidene)-4-(2,5-difluorobenzylxyloxy)-3-chlorobenzohydrazide (9a).

**Fig. 5.5:** ($^1$H NMR spectrum of 9a)

**Fig. 5.6:** ($^{13}$C NMR spectrum of 9a)
(E)-N’-(2,5-dimethoxybenzylidene)-4-(2,5-difluorobenzyloxy)-3-chlorobenzohydrazide (9b).

**Fig. 5.9:** \(^1\)H NMR spectrum of 9b

**Fig. 5.10:** \(^{13}\)C NMR spectrum of 9b
Fig. 5.11: (HRMS spectrum of 9b)

Fig. 5.12: (IR spectrum of 9b)
(E)-4-(2,5-difluorobenzyloxy)-N’-(3,4,5-trimethoxybenzylidene)-3-chlorobenzohydrazide(9d).

Fig. 5.13: $^1$H NMR spectrum of 9d

Fig. 5.14: $^{13}$C NMR spectrum of 9d
Fig. 5.15: (HRMS spectrum of 9d)

Fig. 5.16: (IR spectrum of 9d)
(E)-4-(2,5-difluorobenzyloxy)-N'-(4-ethoxy-3-methoxybenzylidene)-3-chlorobenzohydrazide(9e).

Fig.5.17: ($^1$H NMR spectrum of 9e)

Fig.5.18: (Mass spectrum of 9e)
Fig. 5.19: (IR spectrum of 9e)

(E)-4-(2,5-difluorobenzyloxy)-N’-(3-methoxy-4-propoxybenzylidene)-3-chlorobenzohydrazide (9f).

Fig. 5.20: ($^1$H NMR spectrum of 9f)
Fig. 5.21: $^{13}$C NMR spectrum of 9f

Fig. 5.22: (Mass spectrum of 9f)
Fig. 5.23: (IR spectrum of 9f)

(E)-4-(2,5-difluorobenzyloxy)-N'-(4-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9h).

Fig. 5.24: ($^1$H NMR spectrum of 9h)
Fig. 5.25: ($^{13}$C NMR spectrum of 9h)

Fig. 5.26: (Mass spectrum of 9h)
Fig. 5.27: (IR spectrum of 9h)

(E)-4-(2,5-difluorobenzyloxy)-N’-(3-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9j).

Fig. 5.28: (1H NMR spectrum of 9j)
Fig. 5.29: ($^{13}$C NMR spectrum of 9j)

Fig. 5.30: (Mass spectrum of 9j)
(E)-4-(2,5-difluorobenzyloxy)-N'-(2-(trifluoromethyl)benzylidene)-3-chlorobenzohydrazide (9k).

Fig. 5.31: (IR spectrum of 9j)

Fig. 5.32: ($^1$H NMR spectrum of 9k)
Fig.5.33: $^{13}$C NMR spectrum of 9k

Fig.5.34: Mass spectrum of 9k
Fig. 5.35: (IR spectrum of 9k)

(E)-4-(2,5-difluorobenzyloxy)-N’-(4-fluorobenzylidene)-3-chlorobenzohydrazide (9l).

Fig. 5.36: (\textsuperscript{1}H NMR spectrum of 9l)
Fig. 5.37: ($^{13}$C NMR spectrum of 9l)

Fig. 5.38: (Mass spectrum of 9l)
Note: $^1$H NMR spectra of few compounds contain residual solvent peaks at 1 to 2.4 ppm.
5.7 Conclusion

In summary, synthesized fifteen new hydrazone derivatives \(9a-9o\) and evaluated for their anti-bacterial activities against four selected bacterial strains \(Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes\) and \(Staphylococcus aureus\), at the concentrations 250 µg/mL with reference to the antibiotic drug ampicillin. The results revealed that hydrazone derivatives possessing fluorine moiety \(9h, 9k, \text{ and } 9m\) exhibited excellent antibacterial activity with zone of inhibition 21-24 mm, while the compounds \(9d, 9i\) and \(9o\) having –OCF\(_3\), 3,4,5-tri-OMe and benzo [b] furan moiety displayed good activity towards all the tested bacterial strains. Thus, hydrazone derivative with an appropriate R group may emerge as a good antibacterial agent it may be considered as a promising lead for further design and development of new anti-microbial agents.