CHAPTER-II
Synthesis, Molecular Docking and Antibacterial Evaluation of 
\(N-(6,11\text{-dioxo-6,11-dihydro-5H-benzo}[b]\text{carbazol-2-yl})\text{benzamide Derivatives}

1. Introduction

It was Van Leeuwenhoek who identified the Bacteria in the 1670s following his invention of the microscope. After Van Leeuwenhoek, French scientist Pasteur carried out a number of experiments and demonstrated that specific bacterial strains were crucial to fermentation and that these and other microorganisms were far more widespread than was previously thought. These microorganisms might be the reason for diseases in human beings and animals. Millions of patients suffer from the effect of microorganisms and antibiotics and other antimicrobial drugs have saved them. At the same time bacteria can develop resistance to existing drugs and there is a difficulty to infect the bacteria\(^1\) and there is a need to develop new effective antibiotics and antimicrobial drugs to overcome this problem.\(^2\)

Quinones and heterocyclic quinones are a large class of compounds with diverse biological activity. They are found to be very cheap and easily available, having a wide range of biological applications which includes electron transport and oxidative phosphorylation.\(^3\) Heterocyclic quinones are biologically active\(^4\) and heterocyclic aminoquinones have number of successive biological applications including anticancer,\(^5,31\) antibacterial,\(^6,7\) fungicidic,\(^7,8\) luciferase inhibition,\(^9\) antiproliferative\(^10\) and tuberculostatic effects.\(^11\) In addition, the heterocyclic naphthoquinone derivatives exhibit potent properties like electrochemical capacitance,\(^12\) electrochemical redox\(^{13,32}\) electron mediator\(^{14}\) and electron transfer\(^{15}\) in many biological systems. They are also capable molecules to form complexes with metals.\(^{16}\)

In our previous reports\(^{17,18}\) a few novel quinones and heterocyclic quinones were studied for their fluorescent switching properties. In this report we present the study
of the antibacterial activity of novel \( N-(6,11\text{-dioxo-6,11-dihydro-5H-benzo}[b]\text{carbazol-2yl})\text{benzamide derivatives which is not yet reported in literature. This present investigation deals with the effect of synthesized compounds on some clinically isolated different Gram-positive and Gram-negative bacteria. To understand the interactions of the tested compounds at the active sites of the protein receptors, the molecular docking studies were also preformed and reported in this chapter.}

In the previous chapter, carbazole-6,11-diones with 1,4-naphthoquinone moiety was synthesized and studied for their \textit{in vitro} antibacterial properties. We were motivated and interested to study the biological properties of such molecules. Therefore we synthesized molecules with different functional groups having carbazole moiety. In this chapter \( p\)-phenylene diamine was used as a reagent to construct the molecules with carbazole moiety and they were studied for their \textit{in vitro} antibacterial activities and the results are reported.

2. \textbf{Review of literature}

Synthetic quinones and heterocyclic quinones exhibit promising activity due to their redox potentials. Quinones having hetero atoms inside the ring i.e., heterocyclic moiety show enhanced biological activity like anticancer, antimalarial, antimicrobial and antiviral properties.

2.1. \textbf{Biological properties of synthetic quinones}

The antifungal activity of 5-arylamino- and 6-arylthio-4,7-dioxobenzoselenazole derivatives were reported by Ryu et al.,\textsuperscript{19} in 2005 against different fungal pathogens and most of the synthesized compounds showed potent antifungal activity.
In 2005, Chung et al.,\textsuperscript{20} reported the synthesis of quinoxaline-5,8-dione derivatives and evaluated for their inhibition activity of vascular smooth muscle cell proliferation and the results reveal that the compounds 48 and 53 exhibits better IC\textsubscript{50} of 1 \mu M.

The synthesis of naphthoquinone moiety with heterocyclic skeleton was reported by Kim et al.,\textsuperscript{21} in 2007. These compounds exhibited better cytotoxicity and DNA topoisomerase II inhibitory activities.
In 2007, the same research group (Ryu et al.,)\textsuperscript{22} reported the synthesis of naphthoquinone derivative with nitrogen atoms inside the ring and the results reveals that most of the compounds exhibited potent antifungal activity.

In 2009, Valderrama et al.,\textsuperscript{23} reported the synthesis of novel 7-aminoisoquinoline-5,8-quinone derivatives possessing the heterocyclic moiety. They were studied for their cytotoxic properties against human normal cells (MRC-5), human lung fibroblasts and three human tumor cells: gastric adenocarcinoma (AGS), lung cancer (SK-MES-1) and bladder carcinoma (J82).
3. Experimental

3.1. Materials and methods

Melting points (°C, uncorrected) of all the synthesized compounds were checked in capillary tubes by using a digital melting point apparatus (Labtronics 110, India) and found uncorrected. All the analytical grade chemicals and solvents were purchased from Sigma-Aldrich, Merck and HiMedia India. Progress and completion of all the reactions were monitored by thin layer chromatography (TLC silica gel 0.25 mm, 60 G F254 and eluting solvents were ethyl acetate: hexane 1:9). All the compounds were characterized by FT-IR spectrometer (IR Prestige-21, Shimadzu, Japan) using KBr pellets, ^1^H NMR spectroscopy in DMSO-\textit{d}_6 (500 MHz, Bruker) and ^13^C NMR spectroscopy in DMSO-\textit{d}_6 (125 MHz, Bruker) using tetramethylsilane (TMS) as internal standard. High resolution mass spectra (HRMS-EI) were measured by Electron Ionization (EI) method (Jeol GC-Mate 2). An \textit{in vitro} antibacterial study of all the compounds was studied by agar dilution method. The molecular docking studies of all the synthesized compounds were studied by GLIDE program (version 8.5, Schrodinger, LLC, New York, 2010).

3.2. Procedure for synthesis of 2-(4-aminophenylamino)naphthalene-1,4-dione (8)

A mixture of 1,4-naphthoquinone (1) (10 mmol, 1.58 g) and \textit{para}-phenylene diamine (7) (10 mmol, 1.08 g) was added to absolute ethanol (250 mL) and the mixture was refluxed for 10 h. The contents were cooled to room temperature and the reaction mixture was poured into water containing crushed ice and the precipitate formed was collected by vacuum filtration. The product was dried at 50 °C and recrystalized from acetone.
Fig. 32: $^1$H NMR Spectrum of compound 8
Fig. 33: $^{13}$C NMR Spectrum of compound 8

Black solid; (2.25 g, 85%); mp > 300 ºC; IR (KBr): 1120, 1271, 1354, 1436, 1512, 1568, 1670, 3313; $^1$H NMR (500 MHz, DMSO-$d_6$): 5.23 (s, 2H), 5.86 (s, 1H), 6.62 (d, 2H, $J$ = 8.4 Hz), 7.01 (d, 2H, $J$ = 8.0 Hz), 7.74 (t, 1H, $J$ = 6.4 Hz), 7.82 (t, 1H, $J$ = 6.4 Hz), 7.93 (d, 1H, $J$ = 7.6 Hz), 8.03 (d, 1H, $J$ = 7.6 Hz), 8.96 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 100.2, 114.0, 125.1, 125.2, 125.3, 125.6, 125.7, 125.9, 126.4, 130.4, 132.2, 132.9, 134.7, 146.9, 181.7, 181.7; HRMS (El) $m/z$: Calcd for C$_{16}$H$_{12}$N$_2$O$_2$: 264.2786 Found: 264.2787.
3.3. General procedure for synthesis of \( N-(4-(1,4\text{-dioxo-1,4\text{-dihydropyanthene-2ylamino})phenyl})\text{benzamides (9a-g)}} \)

Different substituted acid chlorides (1 mmol) was added to a solution of 8 (0.264 g, 1 mmol) in acetone (100 mL). After refluxing for 30 min, the reaction mixture was filtered and concentrated \text{in vacuo} to give pure samples of 9a-g which required no further purification.

3.3.1. \( N-(4-(1,4\text{-dioxo-1,4\text{-dihydropyanthene-2ylamino})phenyl})\text{benzamide (9a)}} \)

![9a](image)

\textbf{Fig. 34:} \( ^1\text{H NMR Spectrum of compound 9a}} \)
Fig. 35: $^{13}$C NMR Spectrum of compound 9a

Purple solid; Reaction time 30 minutes (0.345 g, 94%); mp > 300 °C; IR (KBr): 1296, 1409, 1514, 1548, 1600, 1672, 3288 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$: 6.09 (s, 1H), 7.38 (d, 2H, $J = 9.0$ Hz), 7.47-7.56 (m, 4H), 7.59 (t, 1H, $J = 6.0$ Hz), 7.79 (t, 1H, $J = 6.0$ Hz), 7.85 (d, 2H, $J = 6.0$ Hz), 7.94 (t, 1H, $J = 7.0$ Hz), 7.98 (d, 1H, $J = 7.2$ Hz), 8.06 (d, 1H, $J = 8.0$ Hz) 9.23 (s, 1H), 10.36 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$: 102.1, 121.5, 124.5, 125.7, 126.5, 128.1, 128.8, 129.0, 129.7, 130.9, 131.2, 132.0, 133.0, 133.1, 133.3, 133.9, 135.3, 136.9, 146.7, 165.7, 182.1, 182.8; HRMS (EI) m/z: Calcd for C$_{23}$H$_{16}$N$_2$O$_3$: 368.3847 Found: 368.3873.
3.3.2. *N*-(4-(1,4-dioxo-1,4-dihydropthalene-2ylamino)phenyl)-3-methylbenzamide (9b)

Fig. 36: $^1$H NMR Spectrum of compound 9b
Fig. 37: $^{13}$C NMR Spectrum of compound 9b

Purple solid; Reaction time 30 minutes (0.360 g, 94%); mp > 300 ºC; IR (KBr): 1190, 1234, 1296, 1354, 1408, 1512, 1546, 1598, 1666, 3300, 3365 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.41 (s, 3H), 6.09 (s, 1H), 7.37 (d, 2H, $J = 8.5$ Hz), 7.42 (d, 2H, $J = 8.5$ Hz), 7.5-7.80 (m, 4H), 7.84 (t, 2H, $J = 8.0$ Hz), 7.95 (d, 1H, $J = 7.5$ Hz), 8.06 (d, 1H, $J = 7.5$ Hz), 9.23 (s, 1H), 10.31 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 21.4, 102.1, 121.4, 124.5, 125.2, 125.7, 126.5, 128.5, 128.7, 132.6, 135.3, 138.1, 147.0, 166.3, 182.3, 183.3; HRMS (EI) $m/z$: Calcd for C$_{24}$H$_{18}$N$_2$O$_3$: 382.4113 Found: 382.4112.
3.3.3. *N*-\((4-(1,4\text{-dioxo-1,4\text{-dihydrornaphthalene}}-2\text{ylamino})\text{phenyl})-4\text{-methylbenzamide} (9c)*

Fig. 38: $^1$H NMR Spectrum of compound 9c
**Fig. 39:** $^{13}$C NMR Spectrum of compound 9c

Purple solid; Reaction time 30 minutes (0.355g, 94%); mp > 300 °C; IR (KBr): 1118, 1180, 1234, 1355, 1408, 1512, 1595, 1656, 1685, 3271 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 1.18 (s, 3H), 6.02 (s, 1H), 7.31-7.89 (m, 11H), 9.16 (d, 1H, $J = 8.0$ Hz), 10.20 (s, 1H), 12.76 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 20.3, 113.2, 117.0, 122.8, 124.3, 126.8, 132.5, 133.8, 134.8, 135.2, 136.8, 137.7, 138.0, 138.1, 139.2, 140.7, 141.2, 142.5, 143.6, 144.2, 147.6, 166.8, 178.2, 181.0; HRMS (EI) $m/z$: Calcd for C$_{24}$H$_{18}$N$_{2}$O$_{3}$: 382.4113 Found: 382.4113.
3.3.4. \(N-\left(4-(1,4\text{-dioxo-1,4\text{-dihydronaphthalene-2ylamino}})\text{phenyl}\right)\text{-3-nitrobenzamide (9d)}\)

**Fig. 40:** \(^1\text{H NMR Spectrum of compound 9d}\)
Purple solid; Reaction time 30 minutes (0.390 g, 95%); mp > 300 ºC; IR (KBr): 1120, 1238, 1294, 1352, 1408, 1533, 1616, 1672, 3269 cm⁻¹; ¹H NMR (500 MHz, DMSO-d6) δ: 6.11 (s, 1H), 7.40 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 8.5 Hz), 7.94 (d, 2H, J = 8.0 Hz), 8.05 (d, 1H, J = 7.5 Hz), 8.33-8.50 (m, 4H), 8.61 (d, 1H, J = 8.0 Hz), 9.23 (s, 1H), 10.68 (s, 1H); ¹³C NMR (125 MHz, DMSO-d6) δ: 121.7, 122.8, 124.1, 124.5, 125.7, 126.5, 126.6, 127.7, 130.6, 130.8, 130.9, 132.9, 133.0, 133.1, 134.4, 134.6, 135.3, 135.8, 136.3, 136.6, 146.6, 165.9, 182.0, 182.9; HRMS (EI) m/z: Calcd for C₂₃H₁₅N₃O₅: 413.3823 Found: 413.3822.
3.3.5. \(N-(4-(1,4\text{-dioxo}-1,4\text{-dihydronaphthalene-2ylamino})\text{phenyl})\text{-}3,5\text{-dinitrobenzamide (9e)}\)

\[9e\]

**Fig. 42:** \(^1\text{H NMR Spectrum of compound 9e}\)
Fig. 43: $^{13}$C NMR Spectrum of compound $9e$

Purple solid; Reaction time 30 minutes (0.435 g, 95%); mp $> 300 \degree C$; IR (KBr): 1080, 1122, 1159, 1242, 1294, 1344, 1409, 1516, 1543, 1668, 3078, 3267 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$: 6.13 (s, 1H), 7.14 (t, 1H, $J = 8.0$ Hz), 7.23 (t, 1H, $J = 7.5$ Hz), 7.34 (d, 1H, $J = 7.5$ Hz), 7.44 (d, 2H, $J = 8.0$ Hz), 7.78 (d, 1H, $J = 8.5$ Hz), 7.86 (d, 2H, $J = 8.8$ Hz), 7.95 (d, 2H, $J = 8.0$ Hz), 8.06 (s, 1H), 9.26 (s, 1H), 10.96 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$: 121.6, 121.9, 122.5, 122.9, 124.5, 125.7, 126.5, 128.5, 129.3, 129.3, 130.8, 133.0, 133.1, 134.8, 134.8, 135.3, 135.9, 137.8, 148.6, 148.8, 164.4, 182.0, 182.9; HRMS (El) $m/z$: Calcd for C$_{23}$H$_{14}$N$_4$O$_7$: 458.3798 Found: 458.3798.
3.3.6. \( N-(4-(1,4\text{-dioxo}-1,4\text{-dihydronaphthalene-2ylamino})\text{phenyl})\text{acetamide (9f)} \)

![Diagram of compound 9f]

**Fig. 44:** \(^1\)H NMR Spectrum of compound **9f**
Purple solid; Reaction time 30 minutes (0.290 g, 95%); mp > 300 °C; IR (KBr): 1122, 1269, 1294, 1357, 1409, 1523, 1566, 1606, 1683, 3194, 3309 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 2.06 (s, 3H), 6.03 (s, 1H), 7.30 (d, 2H, J = 8.5 Hz), 7.63 (d, 2H, J = 9.0 Hz), 7.77 (t, 1H, J = 7.5 Hz), 7.84 (t, 1H, J = 7.5 Hz), 7.94 (d, 1H, J = 7.5 Hz), 8.05 (d, 1H, J = 7.5 Hz), 9.17 (s, 1H), 10.04 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 24.7, 101.9, 120.1, 124.7, 125.7, 126.5, 130.9, 133.0, 135.3, 171.7, 181.6, 183.0; HRMS (EI) m/z: Calcd for C₁₈H₁₄N₂O₃: 306.3153 Found: 306.3153.
3.3.7. 2-chloro-N-(4-(1,4-dioxo-1,4-dihydonaphthalene-2ylamino)phenyl)acetamide (9g)

Fig. 46: $^1$H NMR Spectrum of compound 9g
Fig. 47: $^{13}$C NMR Spectrum of compound 9g

Purple solid; Reaction time 30 minutes (0.320 g, 94%); mp > 300 ºC; IR (KBr): 989, 1124, 1294, 1359, 1409, 1521, 1564, 1597, 1676, 3076, 3190 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 4.26 (s, 2H), 6.06 (s, 1H), 7.35 (d, 2H, $J = 8.5$ Hz), 7.64 (d, 2H, $J = 8.5$ Hz), 7.76 (t, 1H, $J = 7.5$ Hz), 7.84 (t, 1H, $J = 7.5$ Hz), 7.93 (d, 1H, $J = 8.0$ Hz), 8.05 (d, 1H, $J = 7.5$ Hz), 9.20 (s, 1H), 10.40 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 44.0, 102.1, 120.6, 124.7, 125.7, 126.5, 130.8, 133.0, 133.1, 134.1, 135.3, 136.1, 146.7, 165.0, 182.0, 182.8; HRMS (EI) $m/z$: Calcd for C$_{18}$H$_{13}$ClN$_2$O$_3$: 340.7604 Found: 340.7604.
3.4. General Procedure for synthesis of \( N-(6,11\text{-dioxo}-6,11\text{-dihydro-}5H\text{-benzo}[b]\text{carbazol-2-yl})\text{benzamide derivatives (10a-g)} \)

A mixture of \( 9a-g \) (0.5 mmol) in glacial acetic acid (60 mL) and palladium (II) acetate (0.112 g, 0.5 mmol) were refluxed for 2 h and the reaction mixture was cooled at room temperature and poured into ice cold water. The precipitate was filtered, dried at 60 °C and crystallized from acetone to give compounds \( 10a-g \).

3.4.1. \( N-(6,11\text{-dioxo-}6,11\text{-dihydro-}5H\text{-benzo}[b]\text{carbazol-2-yl})\text{benzamide (10a)} \)

\[ \text{Fig. 48:} \quad ^1\text{H NMR Spectrum of compound 10a} \]
Fig. 49: $^{13}$C NMR Spectrum of compound 10a

Yellow solid; Reaction time 2 hours (0.265 g, 73%); mp $> 300 \, ^\circ$C; IR (KBr): 1010, 1240, 1271, 1375, 1481, 1587, 1647, 3277 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 7.54-7.61 (m, 5H), 7.80-7.89 (m, 4H), 8.02 (d, 2H, $J = 7.0$ Hz), 8.75 (s, 1H), 10.43 (s, 1H), 13.09 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 113.4, 114.2, 122.2, 124.6, 126.4, 126.6, 128.1, 128.8, 132.0, 133.1, 134.6, 135.4, 135.5, 136.3, 137.9, 165.9, 177.8, 180.6; HRMS (EI) $m/z$: Calcd for C$_{23}$H$_{14}$N$_2$O$_3$: 366.3688 Found: 366.3687.
3.4.2. \( N-(6,11\text{-dioxo-6,11-dihydro-5}H\text{-benzo}[b]\text{carbazol-2-yl)-3-methylbenzamide (10b)} \)

Fig. 50: \(^1H\) NMR Spectrum of compound 10b
**Fig. 51:** $^{13}$C NMR Spectrum of compound 10b

Yellow solid; Reaction time 2 hours (0.280 g, 74%); mp > 300 ºC; IR (KBr): 1012, 1238, 1273, 1321, 1377, 1431, 1481, 1535, 1591, 1645, 1668, 2920, 3277 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.43 (s, 3H), 7.43 (d, 2H, $J = 7.0$ Hz), 7.57 (d, 1H, $J = 8.0$ Hz), 7.81 (d, 1H, $J = 7.5$ Hz), 7.83 (d, 1H, $J = 6.0$ Hz), 7.86 (t, 1H, $J = 7.8$ Hz), 7.89 (t, 1H, $J = 8.0$ Hz), 8.10 (d, 2H, $J = 7.0$ Hz), 8.13 (d, 1H, $J = 8.0$ Hz), 8.74 (s, 1H), 10.37 (s, 1H), 13.08 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 21.4, 113.3, 114.2, 117.8, 122.2, 124.6, 125.3, 126.4, 126.6, 128.6, 128.7, 132.5, 133.1, 133.6, 134.6, 135.4, 135.5, 136.3, 137.9, 138.1, 166.0, 177.8, 180.6; HRMS (EI) $m/z$: Calcd for C$_{24}$H$_{16}$N$_2$O$_3$: 380.3954 Found: 380.3954.
3.4.3. \(N\)-(6,11-dioxo-6,11-dihydro-5\(H\)-benzo[\(b\]carbazol-2-yl)-4-methylbenzamide (10c)

Fig. 52: \(^1\)H NMR Spectrum of compound 10c
Fig. 53: $^{13}$C NMR Spectrum of compound 10c

Yellow solid; Reaction time 2 hours (0.278g, 74%); mp > 300 ºC; IR (KBr): 1010, 1273, 1323, 1483, 1527, 1589, 1641, 1668, 2358, 2848, 2918, 3267 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 1.23 (s, 3H), 7.35 (d, 2H, $J = 8.0$ Hz), 7.57 (d, 1H, $J = 8.5$ Hz) 7.81-7.89 (m, 3H), 7.94 (d, 2H, $J = 8.0$ Hz), 8.10 (d, 1H, $J = 7.5$ Hz), 8.15 (d, 1H, $J = 7.5$ Hz), 8.74 (s, 1H), 10.33 (s, 1H), 13.08 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 21.3, 114.7, 115.3, 117.3, 121.3, 124.7, 128.8, 133.2, 134.0, 135.0, 136.3, 137.5, 138.8, 139.0, 140.2, 141.4, 142.3, 144.0, 145.9, 146.1, 147.3, 166.1, 177.0, 178.3; HRMS (EI) m/z: Calcd for C$_{24}$H$_{16}$N$_2$O$_3$: 380.3954 Found: 380.3953.
3.4.4. *N*-(6,11-dioxo-6,11-dihydro-5*H*-benzo[*b*]carbazol-2-yl)-3-nitrobenzamide (10d)

![Chemical structure of compound 10d]

**Fig. 54:** $^{13}$NMR Spectrum of compound 10d
Yellow solid; Reaction time 2 hours (0.300 g, 72%); mp > 300 °C; IR (KBr): 1010, 1240, 1271, 1327, 1485, 1527, 1591, 1668, 2918, 3205 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 7.61 (d, 1H, J = 6.8 Hz), 7.69 (d, 1H, J = 7.5 Hz), 7.83-7.99 (m, 7H), 8.74 (s, 1H), 8.87 (s, 1H), 10.75 (s, 1H), 13.11 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 105.3, 110.4, 113.6, 118.1, 122.1, 122.9, 126.5, 126.6, 130.6, 134.6, 135.7, 136.7, 148.2, 163.7, 177.8, 178.3; HRMS (EI) m/z: Calcd for C₂₃H₁₃N₃O₅: 411.3664; Found: 411.3664.
3.4.5. \( \text{N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)-3,5-dinitrobenzamide (10e)} \)

**Fig. 56:** \(^1\text{H NMR Spectrum of compound 10e}\)
Fig. 57: $^{13}$C NMR Spectrum of compound 10e

Yellow solid; Reaction time 2 hours (0.340 g, 75%); mp > 300 °C; IR (KBr): 1016, 1155, 1246, 1330, 1489, 1541, 1587, 1666, 2918, 3084, 3203, 3390 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 7.18 (d, 1H, $J$ = 5.8 Hz), 7.36 (d, 1H, $J$ = 6.2 Hz), 7.76-8.13 (m, 7H), 8.72 (s, 1H), 11.01 (s, 1H), 13.15 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 100.1, 102.7, 103.1, 104.8, 105.1, 106.7, 107.1, 108.3, 109.1, 110.9, 112.1, 114.5, 121.8, 125.9, 127.1, 128.0, 128.5, 134.8, 148.6, 169.5, 180.2, 181.7; HRMS (EI) $m/z$: Caled for C$_{23}$H$_{12}$N$_4$O$_7$: 456.3639 Found: 456.3639.
3.4.6. \( N-(6,11\text{-dioxo-}6,11\text{-dihydro-}5H\text{-benzo}[b]\text{carbazol-2-yl})\text{acetamide (10f)} \)

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\begin{align*}
\text{Fig. 58: } & \, ^1\text{H NMR Spectrum of compound 10f} \\
\end{align*}
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Fig. 59: $^{13}$C NMR Spectrum of compound 10f

Yellow solid; Reaction time 2 hours (0.230 g, 75%); mp $> 300$ °C; IR (KBr): 1008, 1240, 1273, 1369, 1516, 1589, 1647, 2926, 3442 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 1.23 (s, 3H), 7.51 (d, 1H, $J = 8.5$ Hz), 7.64 (d, 1H, $J = 8.0$ Hz), 7.71 (s, 1H), 7.80-7.90 (m, 4H), 10.09 (s, 1H), 13.02 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 24.4, 91.2, 98.3, 101.3, 109.8, 114.4, 126.5, 133.2, 134.6, 142.8, 143.3, 150.7, 168.9, 180.7, 183.0; HRMS (EI) $m/z$: Calcd for C$_{18}$H$_{12}$N$_2$O$_3$: 304.2994 Found: 304.2994.
3.4.7. 2-chloro-N-(6,11-dioxo-6,11-dihydro-5\textit{H}-benzo[\textit{b}]carbazol-2-yl)acetamide (10g)

**Fig. 60:** $^1$H NMR Spectrum of compound 10g
Yellow solid; Reaction time 2 hours (0.250 g, 74%); mp > 300 °C; IR (KBr): 1006, 1273, 1375, 1531, 1587, 1672, 3275, 3739 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 4.30 (s, 2H), 7.51 (d, 1H, J = 6.5 Hz), 7.63 (d, 1H, J = 7.5 Hz), 7.80-7.90 (m, 2H), 8.09-8.16 (m, 2H), 10.08 (s, 1H), 10.47 (s, 1H), 13.08 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 69.0, 101.3, 102.6, 103.1, 104.7, 105.8, 110.3, 114.1, 118.2, 121.3, 126.8, 130.2, 132.0, 137.8, 141.2, 163.2, 171.4, 178.2; HRMS (EI) m/z: Calcd for C₁₈H₁₁ClN₂O₃: 338.7445 Found: 338.7445.
3.5. Molecular docking studies

To understand the interaction of all the synthesized molecules (8, 9a-g, 10a-g) with *Bacillus subtilis*, the crystal structure of ymaH from *Bacillus subtilis*\textsuperscript{26} (PDB ID: 3HSB) was downloaded from protein data bank and the molecular docking studies were performed using the GLIDE program\textsuperscript{27} (version 8.5, Schrodinger, LLC, New York, 2010). To analyze the docking results and to execute the protocol, the maestro user interface (version 8.5, Schrodinger, LLC, New York, 2010) was employed and the validation of the protocol was evaluated by redocking. ymaH (PDB ID: 3HSB) was selected for docking studies as a reference sample and was prepared for docking through protein preparation wizard. The structures of compounds 8, 9a-g, 10a-g were sketched using ACD/chemsketch (Freeware version). GLIDE grid generation wizard has been used to define the docking space. The docking was performed using XP (Extra Precision mode) docking protocol.

3.6. *In vitro* antibacterial activity

All the synthesized compounds were studied for their antibacterial activity against clinically isolated two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and five Gram-negative bacilli (*Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeruginosa*) using conventional agar-dilution method.\textsuperscript{28,29} The minimum inhibitory concentration (MIC) values were calculated by comparison with Sparfloxacin and Norfloxacin which were used as the standard antibacterial drugs and they are presented in Table 4. All the cultures were prepared by Muller Hinton agar and the turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standard by preparing bacterial suspension of 3–5 well-isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted 1000-fold to get an inoculums size of 1.5 X 10\textsuperscript{5} CFU/mL. The synthesized compounds and standard antibacterial drugs (50 mg) were dissolved in Dimethyl formamide (DMF) (0.5 mL) and the solution was diluted with water (4.5 mL) to get a stock solution of 10,000 mg/L of each compound. Further
progressive double dilution with Muller Hinton broth was performed to obtain the required concentrations of 2500-0.7 µg/mL. To ensure that the solvent had no effect on the bacterial growth, a control test was performed with a test medium supplemented with DMF at the same dilutions as used in the experiment.

In each micro well inoculated with 75 µL of the serial dilutions, 75 µL of the bacterial suspension was added in a series of 12 micro wells. Incubation of the cultures overnight at 37 ºC was done and the growth measured. The MIC of the test compounds and the standard control drugs are tabulated in Table 4.

4. Results and discussion

4.1. Chemistry

In our previous report, simple “off-on-off” chemical and electrochemical fluorescent switches were successfully demonstrated. The compound 8 was synthesized by the nucleophilic amino addition reaction of equal mole concentrations of 1,4-naphthoquinone and p-phenylene diamine in the presence of absolute ethanol. The mixture was refluxed for 10 h and the yield obtained was 85%. In a previous report the same compound (8) was synthesized by amino substitution in the presence of absolute ethanol at room temperature. The product was obtained after 96 h of stirring at room temperature and the percentage yield of compound 8 was not given. In another report the same reaction was carried out in the presence of water and glacial acetic acid mixture under reflux condition and the yield reported was between 49-62%.
Scheme 2: The synthesis of 2-(4-aminophenylamino)naphthalene-1,4-dione (8), N-(4-(1,4-dioxo-1,4-dihyronaphthalene-2ylamino)phenyl)benzamides (9a-g) and N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2yl)benzamides (10a-g).

In one of our previous study we reported\textsuperscript{17} the nucleophilic amino addition and substitution reactions with 1,4-quinone moiety in the presence of ethanol, water and solvent free microwave system. Based on the above literature we attempted the amino substitution reaction with 1,4-naphthoquinone in the presence of water as solvent and the reaction was not successful. So we resorted to the conventional method in ethanol.
A number of inseparable compounds were formed when water was used as the solvent and the yield was low (25%) compared to ethanol mediated amino addition. In the second step, the compound 8 was benzyolated with different substituted aromatic acid chlorides to give compounds 9a-g and finally these compounds were subjected to intramolecular carbon-carbon bond linkage by palladium (II) acetate in the presence of glacial acetic acid to give the products 10a-g and the product yield was between 72-75%. The percentage of yield for compounds (10a-g) is comparatively very good. In the reported work\textsuperscript{24} no information about the product yield was given and in another report\textsuperscript{25} for the palladium catalyzed reaction, the yields were between 18-62%.

4.2. Biology

4.2.1. Molecular docking studies of quinone derivatives

To understand the interaction of bacterial protein receptor with synthesized molecules (8, 9a-g, 10a-g) the crystal structure of ymaH from \textit{Bacillus subtilis} was downloaded from protein data bank and studied with the glide program (See figures 62-64). The entire glide, E model scores and hydrogen bond interactions are compared with the MIC of the tested compounds against \textit{Bacillus subtilis} and are presented in table 3.
Table 3: Molecular docking studies of fifteen analogues taken for study with *Bacillus subtilis* (BS) (PDB ID: 3HSB)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Glide score</th>
<th>E model score (kcal/mol)</th>
<th>No. of Hydrogen bonds interactions</th>
<th>MIC of BS (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>-4.92</td>
<td>-34.71</td>
<td>2 (ASP 269, HIS 180)</td>
<td>33.9</td>
</tr>
<tr>
<td>9a</td>
<td>-5.33</td>
<td>-57.97</td>
<td>1 (LEU 142)</td>
<td>52.8</td>
</tr>
<tr>
<td>9b</td>
<td>-4.47</td>
<td>-60.14</td>
<td>2 (HIE 268, LYS 179)</td>
<td>52</td>
</tr>
<tr>
<td>9c</td>
<td>-5.86</td>
<td>-63.79</td>
<td>1 (GLN 63)</td>
<td>26.1</td>
</tr>
<tr>
<td>9d</td>
<td>-5.05</td>
<td>-63.29</td>
<td>1 (ASP 269)</td>
<td>29.3</td>
</tr>
<tr>
<td>9e</td>
<td>-3.86</td>
<td>-75.11</td>
<td>Hydrophobic interaction</td>
<td>52</td>
</tr>
<tr>
<td>9f</td>
<td>-5.16</td>
<td>-49.42</td>
<td>1 (ASP 269)</td>
<td>*</td>
</tr>
<tr>
<td>9g</td>
<td>-5.78</td>
<td>-60.78</td>
<td>2 (GLN 63, GLN 208)</td>
<td>31.2</td>
</tr>
<tr>
<td>10a</td>
<td>-3.26</td>
<td>-63.54</td>
<td>1 (ASN 273)</td>
<td>524</td>
</tr>
<tr>
<td>10b</td>
<td>-3.96</td>
<td>-70.62</td>
<td>1 (ASN 273)</td>
<td>457</td>
</tr>
<tr>
<td>10c</td>
<td>-3.96</td>
<td>-66.01</td>
<td>1 (ASN 273)</td>
<td>8.8</td>
</tr>
<tr>
<td>10d</td>
<td>-3.16</td>
<td>-72.76</td>
<td>1 (ASN 273)</td>
<td>21.3</td>
</tr>
<tr>
<td>10e</td>
<td><strong>-4.24</strong></td>
<td><strong>-72.39</strong></td>
<td>2 (ASP 274, TRP 58)</td>
<td><strong>6</strong></td>
</tr>
<tr>
<td>10f</td>
<td>-5.77</td>
<td>-44.69</td>
<td>1 (GLN 208)</td>
<td>94</td>
</tr>
<tr>
<td>10g</td>
<td>-4.87</td>
<td>-52.74</td>
<td>1 (GLN 63)</td>
<td>83.1</td>
</tr>
<tr>
<td>Sparfloxacin*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.76</td>
</tr>
<tr>
<td>Norfloxacin*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
</tbody>
</table>

-Docking studies not carried out; *No inhibition observed; Bold letters indicate better activity glide score against *Bacillus subtilis* (BS); *Standard antibacterial drugs.*
Fig. 62: Docking model structure of compound 9c into the ymaH binding pocket.
Fig. 63: Docking model structure of compound 9g into the ymaH binding pocket.

Fig. 64: Docking model structure of compound 10e into the ymaH binding pocket.
4.2.2. *In vitro* antibacterial studies of quinone derivatives

All the synthesized compounds were tested for their *in vitro* antibacterial property against two Gram-positive and five Gram-negative bacteria. All the compounds (8, 9a-g, 10a-g) exhibited good antibacterial activity against Gram-negative bacteria of *Klebsiella pneumoniae* than the standard drugs used (Sparfloxacin and Norfloxacin). The compound 10e exhibits good activity against most of the Gram-positive and Gram-negative microorganisms due to the presence of the two nitro groups at the third and fifth position of the aromatic system of the benzoyl unit. Compound 9e exhibits better activity against *Escherichia coli* (98 µg/mL) than Sparfloxacin (156.3 µg/mL) and Norfloxacin (625 µg/mL). Compound 8 (1.2 µg/mL) exhibits better activity against *Proteus vulgaris* than the standard drug Sparfloxacin (4.8 µg/mL). Compound 10c (227 µg/mL) exhibits better antibacterial activity against *Salmonella typhi* than Sparfloxacin (2500 µg/mL) and Norfloxacin (627 µg/mL). Compound 10a (32 µg/mL), 10b (27 µg/mL), 10c (23 µg/mL) exhibits better antibacterial activity against *Pseudomonas aeruginosa* than the standards of Sparfloxacin (156.3 µg/mL) and Norfloxacin (39.06 µg/mL). Compounds 9b (0.4 µg/mL) and 9e (0.4 µg/mL) exhibited very good antibacterial activity due to the presence of methyl and nitro functional groups, among all the molecules synthesized against *Staphylococcus aureus*, even better than the Sparfloxacin (4.87 µg/mL) and Norfloxacin (39.06 µg/mL). Standard drug Norfloxacin could not exhibit any activity against *Bacillus subtilis* and *Proteus vulgaris* microorganisms. Compound 9f did not exhibit any inhibition against *Bacillus subtilis, Escherichia coli, Proteus vulgaris* and compound 10d did not exhibit any inhibition against *Escherichia coli.*
Pharmacophoric features of the title compounds (10a-g).

With respect to the structure-activity relationship of the synthesized molecules, compounds having electron withdrawing and electron donating nature functional groups (-CH₃, -NO₂, -Cl) exhibit better antibacterial activity. The compound 10e exhibits better antibacterial activity against *B. subtilis* with a MIC of 6 µg/mL due to the presence of dinitro functional group in the aromatic system of the naphthoquinone nucleus. Compounds 9b and 9e exhibits better antibacterial property among all the molecules studied against *S. aureus* with a MIC of 0.4 µg/mL, due to the presence of electron donating and electron withdrawing nature of functional groups (-CH₃, -NO₂). The compounds without zinc binding region or metal binding region in the molecule exhibits lesser antibacterial activity.
Table 4: *In vitro* antibacterial activity of synthesized compounds against Gram-positive and Gram-negative bacteria (MIC in µg/mL).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>B. subtilis</em> (Clinical isolated)</td>
</tr>
<tr>
<td>8</td>
<td>33.9</td>
</tr>
<tr>
<td>9a</td>
<td>52.8</td>
</tr>
<tr>
<td>9b</td>
<td>52</td>
</tr>
<tr>
<td>9c</td>
<td>26.1</td>
</tr>
<tr>
<td>9d</td>
<td>29.3</td>
</tr>
<tr>
<td>9e</td>
<td>52</td>
</tr>
<tr>
<td>9f</td>
<td>*</td>
</tr>
<tr>
<td>9g</td>
<td>31.2</td>
</tr>
<tr>
<td>10a</td>
<td>524</td>
</tr>
<tr>
<td>10b</td>
<td>457</td>
</tr>
<tr>
<td>10c</td>
<td>8.8</td>
</tr>
<tr>
<td>10d</td>
<td>21.3</td>
</tr>
<tr>
<td>10e</td>
<td>6</td>
</tr>
<tr>
<td>10f</td>
<td>94</td>
</tr>
<tr>
<td>10g</td>
<td>83.1</td>
</tr>
<tr>
<td>Sparfloxacina</td>
<td>9.76</td>
</tr>
<tr>
<td>Norfloxacina</td>
<td>*</td>
</tr>
</tbody>
</table>

*No inhibition observed; *Standard antibacterial drugs; Lower MIC values indicate higher antimicrobial activity; Bold letters indicate that the best activity among all compounds studied.

5. Conclusions

In summary, a new series of novel N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2yl)benzamide derivatives were synthesized and characterized by FT-IR, $^1$H, $^{13}$C NMR and high resolution mass (HRMS-EI) spectral analyses. All the molecules were studied for their interactions with ymaH by molecular docking protocol. Among the tested molecules, compound 9e exhibited a good glide score value of -5.86 with e model value of -63.79. *In vitro* antibacterial activity of the tested compounds shows improved activity against all the microorganisms used. In particular compound 9e exhibits marked activity against two microorganisms. The compounds 9b and 9e (0.4 µg/mL) exhibits good activity against *Staphylococcus aureus* than the standard antibacterial drugs Sparfloxacina (4.87 µg/mL) and Norfloxacina (39.06 µg/mL) used.
References


