CHAPTER-III
Synthesis, Molecular Docking and Antibacterial Evaluation of
6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-
benzo[a]phenoxazin-5-one Derivatives

1. Introduction

Quinones are widespread in nature and many drugs have been formulated based on the quinone moiety such as anthracyclines, daunorubicin, doxorubicin, mitomycin, mitoxantrones and saintopin which are used for solid cancer therapy. Heterocyclic quinones play an important role in biological functions and hence they have stimulated basic chemical research. Quinones are important not only as pigments but also as drugs. 1,4 naphthoquinone moiety with electron donating or accepting substituent generates radical anion to form a redox couple and this property is responsible for these compounds to catalytically cycle and generate radicals (hydrogen peroxide and superoxide) to damage cells. 1,4-Naphthoquinone structure is commonly present in different natural products and it leads to various biological activities including anticancer, vascular smooth cell proliferation, Topoisomerase I, antiproliferative, antifungal, antibacterial, antifeedant and tuberculostatic effects. Molecule with DNA intercalating property should possess four coplanar rings. The molecule must also have a para-conjugated quinone ring with nitrogen atoms, which will help it to form a hydrogen bond with DNA. The structure activity relationship of heterocyclic quinones containing nitrogen atoms is very important for the biological properties.

From our previous investigations with carbazole-6,11-diones contains 1,4-naphthoquinone moiety, we were motivated and interested to synthesize varieties of naphthoquinone based heterocycles for biological applications. In this connection we attempted and synthesized phenoxazin-5-one derivatives which possess a number of biological properties. In our recent report in 2014 we synthesized phenoxazin-5-one
derivatives and they were found to exhibit better \textit{in vitro} antibacterial and cytotoxicity activities.

  In our present study we synthesized novel 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenoxazin-5-one derivatives (15a-h) which are not yet reported to the best of our knowledge. The molecular docking studies of all the synthesized molecules were performed against \textit{Bacillus subtilis} (PDB ID: 3HSB) and reported in this chapter. \textit{In vitro} antibacterial activities of synthesized compounds against clinically isolated different Gram-positive and Gram-negative bacteria were also studied and are reported in this chapter.

2. Review of literature

  The 2,3-dichloro-1,4-naphthoquinone derivatives possess number of biological activities and attracts the interest in quinone research. The derivatives exhibit not only biological but also electrochemical redox, organic light emitting diodes (OLED) and organic fluorescent dyes properties.

2.1. Synthesis of 2,3-dichloro-1,4-naphthoquinone derivatives

  In 1963, Vanallan et al.,\textsuperscript{22} reported the synthesis of polynuclear heterocycles from 2,3-dichloro-1,4-naphthoquinone with different substituted aromatic amines by a nucleophilic substitution reaction and further leading to the formation of phenazine-6,11-diones on reacting it with sodium azide.
In the same year (1963) Vanallan et al.,\textsuperscript{23} also reported the synthesis of a phenoxazin moiety namely 6-chloro-benzo[a]phenoxazin-5-one by reacting 2,3-dichloro-1,4-naphthoquinone with 2-aminophenol in ethanol and pyridine.

In 1969, Carroll et al.,\textsuperscript{24} have reported the preparation of some sulfonamide and diaminodiphenyl sulfone analogs of 1,4-naphthoquinone derivatives followed by the catalytic reaction with \textit{N},\textit{N}-diethylaniline in ethanol and the percentage of yield of the product was about 72%.
In the same year 1969, Prescott\textsuperscript{25} synthesized 64 amine derivatives of 1,4-naphthoquinone and tested for their antimalarial activity against \textit{Plasmodium berghei} infection in mice. Two of them, bis[2-chloro-1,4-naphthoquinone-3,3'-sulfonylbis(phenylenimine)] (76) and \textit{N}-4-(2-chloro-1,4-dihydro-1,4-dioxo-3-naphthyl)sulfanilamide (77) showed potent antimalarial activity.

In 1991, Lin et al.,\textsuperscript{26} synthesized and studied the antimalarial activity of a series of 2-aziridinyl and 2,3-bis(aziridinyl)-1,4-naphthoquinonyl sulfonate and acylate derivatives against \textit{Plasmodium falciparum}, Vietnam Smith strain and the compounds 2-aziridinyl-1,4-naphthoquinon-5-yl-p-tert-butylbenzenesulfonate (78), \textit{p}-ethylbenzenesulfonate (79) and 3',4',5'-trimethoxybenzoyl ester (80) showed remarkable antimalarial activity with a IC\textsubscript{50} values of 2.4×10\textsuperscript{-8}, 9.6×10\textsuperscript{-8} and 16×10\textsuperscript{-7} M, respectively.
Tandon et al.,\textsuperscript{27} in 2009 reported a green synthesis of different classes of amides of 2,3-dichloro-1,4-naphthoquinone derivatives by using water as solvent and the yield of the product was between obtained 98-100 % with in a short period of time.

\textbf{Scheme 9}

In the same year of 2009, Tandon et al.,\textsuperscript{28} reported the phenothiazine moiety of 2,3-dichloro-1,4-naphthoquinone derivatives and they evaluated those compounds for their antiproliferative and antifungal properties and most of the compounds exhibited good activity.
3. Experimental

3.1. Materials and methods

Melting points (°C, uncorrected) of the synthesized compounds were checked in open capillary tubes using a digital auto melting point apparatus (Labtronics 110, India) and found uncorrected. All the chemicals and solvents were purchased from Sigma-Aldrich, Merck and Himedia, India. Purity of all the products was checked by thin layer chromatography on a TLC silica gel 60 F254 using eluting solvents such as ethyl acetate and hexane (1:1). The synthesized compounds were purified by column chromatography using column silica gel 100-200 mesh (ethyl acetate: hexane 1:2). All the compounds were characterized by employing a FT-IR spectrometer (IR Prestige-21, Shimadzu, Japan) using KBr pellets. $^1$H NMR spectroscopy in DMSO-$d_6$ (500 MHz, Bruker), $^{13}$C NMR spectroscopy in DMSO-$d_6$ (125 MHz, Bruker) using tetramethylsilane (TMS) as internal standard was also carried out. The coupling constants ($J$ values) are reported in Hz. High-resolution mass spectra (HRMS-EI) was measured by Electron Ionization (EI) method (Jeol GC-Mate 2). Molecular docking studies of all the synthesized compounds were accomplished by GLIDE program.
and the entire glide scores are reported in kcal/mol. The \textit{in vitro} antibacterial study was carried out by agar dilution method and the MIC values were calculated for the tested compounds.

3.2. Synthesis of 2-(4-(4-aminophenylsulfonyl)phenylamino)-3-chloronaphthalene-1,4-dione (13)\textsuperscript{29}

A mixture of 2,3-dichloro-1,4-naphthoquinone (11) (2.270 g, 10 mmol) and 4-aminophenyl sulfone (2) (2.048 g, 10 mmol) was added to distilled water (800 mL) and refluxed for 2 h. The reaction mixture was cooled to room temperature. The red precipitate formed was separated by vacuum filtration, washed with hot water (200 mL), dried at 80 °C and crystallized from 95% ethyl alcohol to give compound 13 (3.850 g, 88%) as red crystals.

Fig. 65: \textsuperscript{1}H NMR Spectrum of compound 13
Red crystals; mp-239.5-240.5 °C; IR (KBr): 1259, 1317, 1516, 1585, 1627, 1676, 1699, 2312, 3286, 3707 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 6.12 (s, 2H), 6.60 (d, 2H, J = 5.2 Hz), 7.17 (d, 2H, J = 4.8 Hz), 7.52 (d, 2H, J = 6.8 Hz), 7.70 (d, 2H, J = 6.8 Hz), 7.80 (t, 1H, J = 7.2 Hz), 7.85 (t, 1H, J = 7.0 Hz), 8.02 (d, 1H, J = 7.2 Hz), 8.04 (d, 1H, J = 7.4 Hz), 9.50 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 112.9, 119.0, 122.1, 125.9, 126.1, 126.5, 126.6, 129.1, 130.4, 131.6, 133.4, 134.6, 137.0, 142.5, 143.1, 153.3, 176.9, 179.7; HRMS (EI) m/z: Calcd for C₂₂H₁₅ClN₂O₄S: 438.8835 Found: 438.8832.
3.3. Synthesis of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenoxazin-5-one (14)

The compound 13 (2.190 g, 5 mmol) was added to the solution of 2-aminophenol (0.550 g, 5 mmol) in 95% of ethyl alcohol (500 mL). The catalytic amount of anhydrous potassium carbonate was added to the reaction mixture and it was refluxed for 2 h. The reaction mixture was cooled to room temperature and poured into crushed ice, the dark blue precipitate formed was filtered by vacuum filtration and the precipitate was washed with distilled water (200 mL), dried at 80 °C and crystallized from dried acetone to give compound 14 (1.930 g, 78%) as dark blue crystals.

![1H NMR Spectrum of compound 14](image)

**Fig. 67:** $^1$H NMR Spectrum of compound 14
Fig. 68: $^{13}$C NMR Spectrum of compound 14

Blue crystals; mp $> 300$ °C; IR (KBr): 1105, 1145, 1315, 1365, 1514, 1591, 1629, 3292, 3363, 3506 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 6.08 (s, 2H), 6.61 (d, 2H, $J = 7.8$ Hz), 6.95 (d, 2H, $J = 8.5$ Hz), 7.37 (t, 1H, $J = 8.0$ Hz), 7.44 (t, 1H, $J = 8.5$ Hz), 7.47 (d, 2H, $J = 6.8$ Hz), 7.57 (d, 2H, $J = 7.2$ Hz), 7.82 (t, 1H, $J = 8.5$ Hz), 7.85 (d, 1H, $J = 7.2$ Hz), 7.87 (t, 1H, $J = 7.5$ Hz), 8.20 (d, 1H, $J = 7.5$ Hz), 8.57 (s, 1H), 8.67 (d, 1H, $J = 8.0$ Hz), 8.68 (d, 1H, $J = 7.8$ Hz); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 113.3, 115.8, 117.2, 119.0, 124.8, 125.8, 126.0, 127.7, 127.8, 129.2, 129.8, 130.8, 131.1, 131.8, 132.3, 132.6, 132.9, 139.9, 144.1, 147.4, 153.5, 179.7; HRMS (EI) $m/z$: Calcd for C$_{28}$H$_{19}$N$_3$O$_4$S: 493.5331 Found: 493.5329.
3.4. General procedure for synthesis of *N*-(*4-(4-(5-oxo-5*H*-benzo[*a*]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamides (15a-h)

Substituted acid chlorides (1 mmol) was added to a solution of 14 (0.493 g, 1 mmol) in acetone (100 mL). After refluxing for 30 min, the reaction mixture was filtered and concentrated *in vacuo* to give pure samples of 15a-h which required no further purification.

3.4.1. *N*-(*4-(4-(5-oxo-5*H*-benzo[*a*]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamide (15a)

![Fig. 69: 1H NMR Spectrum of compound 15a](Image)
Brown solid; Reaction time 30 minutes (0.597 g, 97%); mp > 300 °C; IR (KBr): 1107, 1145, 1259, 1319, 1409, 1517, 1587, 1631, 1693, 2314, 3327 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 6.93 (d, 2H, J = 8.5 Hz), 7.40-8.02 (m, 17H), 8.20 (d, 1H, J = 7.5 Hz), 8.70 (d, 1H, J = 8.0 Hz), 10.63 (s, 1H), 12.94 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 102.1, 121.5, 124.5, 125.7, 126.5, 128.1, 128.8, 129.0, 129.7, 131.2, 133.0, 133.1, 133.3, 133.9, 135.3, 135.3, 136.9, 146.7, 167.7, 182.8; HRMS (EI) m/z: Calcd for C₃₅H₂₃N₃O₅S: 597.6392 Found: 597.6389.

Fig. 70: ¹³C NMR Spectrum of compound 15a
3.4.2. 3-methyl-N-(4-(4-(5-oxo-5H-benzo[a]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamide (15b)

Fig. 71: $^1$H NMR Spectrum of compound 15b
Brown solid; Reaction time 30 minutes (0.600 g, 98%); mp > 300 °C; IR (KBr): 1107, 1145, 1317, 1516, 1587, 3334 cm⁻¹; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\): 2.36 (s, 3H), 6.80 (d, 2H, \(J = 7.2\) Hz), 6.82 (d, 2H, \(J = 8.0\) Hz), 7.01 (d, 1H, \(J = 7.8\) Hz), 7.37-7.47 (m, 3H), 7.67 (d, 1H, \(J = 8.0\) Hz), 7.73 (t, 4H, \(J = 6.8\) Hz), 7.84-7.93 (m, 3H), 7.99 (d, 2H, \(J = 7.0\) Hz), 8.20 (d, 1H, \(J = 8.0\) Hz), 8.68 (d, 1H, \(J = 7.5\) Hz), 10.58 (s, 1H), 12.86 (s, 1H); \(^13\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\): 21.2, 117.1, 120.5, 125.4, 126.9, 128.3, 128.4, 128.7, 128.8, 128.9, 130.1, 131.1, 133.9, 134.8, 137.2, 138.3, 143.7, 167.8, 182.8; HRMS (EI) \(m/z\): Calcd for C\(_{36}\)H\(_{25}\)N\(_3\)O\(_5\)S: 611.6658 Found: 611.6656.

Fig. 72: \(^{13}\)C NMR Spectrum of compound 15b
3.4.3. 4-methyl-N-(4-(4-(5-oxo-5H-benzo[a]phenoxazin-6-ylamino)phenylsulfonxyl)phenyl)benzamide (15c)

Fig. 73: $^1$H NMR Spectrum of compound 15c
Brown solid; Reaction time 30 minutes (0.590 g, 96%); mp > 300 ºC; IR (KBr): 1107, 1149, 1317, 1409, 1508, 1589, 1631, 1687, 3311 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 2.37 (s, 3H), 6.93 (d, 2H, J = 8.0 Hz), 6.99 (d, 2H, J = 7.0 Hz), 7.29 (d, 1H, J = 8.0 Hz), 7.35 (d, 2H, J = 8.0 Hz), 7.38 (t, 1H, J = 7.5 Hz), 7.45 (t, 1H, J = 8.0 Hz), 7.67 (d, 2H, J = 7.0 Hz), 7.83-7.94 (m, 5H), 8.00 (d, 2H, J = 8.0 Hz), 8.20 (d, 1H, J = 8.2 Hz), 8.68 (d, 1H, J = 7.5 Hz), 10.54 (s, 1H), 12.77 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 21.5, 117.1, 120.5, 124.8, 128.3, 128.4, 129.4, 129.5, 129.7, 131.2, 131.9, 132.3, 132.9, 137.1, 143.4, 144.1, 167.7, 182.8; HRMS (EI) m/z: Calcd for C₃₆H₂₅N₃O₅S: 611.6658 Found: 611.6654.
3.4.4. 3-nitro-N-(4-(4-(5-oxo-5\textit{H}-benzo[\textit{a}]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamide (15d)

Fig. 75: $^1$H NMR Spectrum of compound 15d
Fig. 76: $^{13}$C NMR Spectrum of compound 15d

Brown solid; Reaction time 30 minutes (0.620 g, 97%); mp > 300 ºC; IR (KBr): 1107, 1145, 1265, 1319, 1350, 1537, 1587, 1703, 2312, 3340 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-d6) δ: 6.95 (d, 1H, $J = 8.0$ Hz), 6.99 (d, 2H, $J = 7.5$ Hz), 7.39 (t, 1H, $J = 7.5$ Hz), 7.46 (t, 1H, $J = 7.5$ Hz), 7.68 (d, 2H, $J = 7.5$ Hz), 7.81-7.95 (m, 2H), 8.01 (d, 2H, $J = 7.5$ Hz), 8.20 (d, 1H, $J = 8.0$ Hz), 8.35 (d, 1H, $J = 8.0$ Hz), 8.40 (d, 1H, $J = 7.5$ Hz), 8.46 (d, 1H, $J = 6.5$ Hz), 8.62 (d, 2H, $J = 6.8$ Hz), 8.68 (t, 1H, $J = 7.5$ Hz), 8.79 (t, 1H, $J = 7.0$ Hz), 8.80 (d, 1H, $J = 6.9$ Hz), 10.94 (s, 1H), 12.60 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-d6) δ: 116.0, 117.1, 120.9, 123.0, 124.1, 128.4, 134.8, 144.1, 148.6, 160.5, 183.7; HRMS (EI) m/z: Calcd for C$_{35}$H$_{22}$N$_4$O$_7$S: 642.6367 Found: 642.6365.
3.4.5. 4-nitro-\(N\)-(4-(4-(5-oxo-5\(H\)-benzo[\(a\])phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamide (15e)

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\begin{align*}
\text{Fig. 77: } ^1H \text{ NMR Spectrum of compound 15e}
\end{align*}
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Fig. 78: $^{13}$C NMR Spectrum of compound 15e

Brown solid; Reaction time 30 minutes (0.610 g, 95%); mp > 300 °C; IR (KBr): 1107, 1151, 1319, 1346, 1523, 1587, 1625, 1680, 2312, 3508 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 6.94 (d, 1H, $J = 8.0$ Hz), 6.99 (d, 2H, $J = 8.2$ Hz), 7.39 (t, 1H, $J = 8.0$ Hz), 7.46 (t, 1H, $J = 8.0$ Hz), 7.67 (d, 2H, $J = 8.5$ Hz), 7.84-8.02 (m, 5H) 8.17-8.22 (m, 4H), 8.32 (d, 2H, $J = 7.2$ Hz), 8.38 (d, 2H, $J = 8.0$ Hz), 8.68 (s, 1H), 10.94 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 117.1, 120.8, 124.0, 124.2, 128.4, 128.5, 129.8, 130.2, 131.1, 164.9, 179.8; HRMS (El) m/z: Calcd for C$_{35}$H$_{22}$N$_4$O$_7$S: 642.6367 Found: 642.6364.
3.4.6. 3,5-dinitro-N-(4-(4-(5-oxo-5H-benzo[a]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamide (15f)

Fig. 79: $^1$H NMR Spectrum of compound 15f
Brown solid; Reaction time 30 minutes (0.655 g, 96%); mp > 300 °C; IR (KBr): 1105, 1149, 1317, 1404, 1537, 1587, 1625, 1693, 3080, 3315 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) δ: 6.96 (d, 1H, J = 7.8 Hz), 7.00 (d, 1H, J = 8.2 Hz), 7.40 (t, 1H, J = 7.8 Hz), 7.47 (t, 1H, J = 8.0 Hz), 7.68 (d, 2H, J = 8.5 Hz), 7.85-7.98 (m, 4H), 8.02 (d, 2H, J = 8.0 Hz), 8.21 (d, 1H, J = 7.5 Hz), 8.69 (d, 1H, J = 7.8 Hz), 8.90 (d, 2H, J = 8.0 Hz) 9.01-9.03 (m, 2H), 9.16 (d, 2H, J = 6.8 Hz), 11.18 (s, 1H); ¹³C NMR (125 MHz, DMSO-d₆) δ: 113.3, 115.8, 117.2, 119.0, 124.8, 125.8, 126.0, 127.7, 127.8, 129.2, 129.8, 130.8, 131.1, 131.8, 132.3, 132.6, 132.9, 139.9, 144.1, 147.4, 153.5, 179.7; HRMS (EI) m/z: Calcd for C₃₅H₂₁N₅O₉S: 687.6343 Found: 687.6332.
3.4.7. \( N-(4-(4-(5\text{-oxo-5H-} \text{benzo}[\text{a}] \text{phenoxazin-6-ylamino})\text{phenylsulfonyl})\text{phenyl})\text{acetamide (15g)} \)

\[ \text{Fig. 81: } ^1\text{H NMR Spectrum of compound 15g} \]
Brown solid; Reaction time 30 minutes (0.510 g, 95%); mp > 300 °C; IR (KBr): 1147, 1259, 1317, 1516, 1585, 1627, 1676, 1699, 2312, 3286, 3707 cm⁻¹; ¹H NMR (500 MHz, DMSO-d6) δ: 2.08 (s, 3H), 6.91 (d, 1H, J = 8.0 Hz), 6.97 (d, 2H, J = 7.8 Hz), 7.39 (t, 1H, J = 8.2 Hz), 7.44 (t, 1H, J = 7.5 Hz), 7.64 (d, 2H, J = 8.2 Hz), 7.76 (d, 2H, J = 8.2 Hz), 7.81 (d, 1H, J = 6.8 Hz), 7.84 (t, 1H, J = 7.5 Hz), 7.91 (t, 1H, J = 7.5 Hz), 8.20 (d, 2H, J = 8.5 Hz), 8.67 (s, 1H), 8.68 (d, 2H, J = 7.8 Hz), 10.35 (s, 1H); ¹³C NMR (125 MHz, DMSO-d6) δ: 24.6, 115.9, 117.1, 118.7, 119.2, 125.8, 128.4, 130.8, 131.8, 132.3, 132.9, 136.6, 144.1, 148.6, 169.5, 180.1; HRMS (EI) m/z: Calcd for C₃₀H₂₁N₃O₅S: 535.5698 Found: 535.5695.
3.4.8. 2-chloro-N-(4-(4-(5-oxo-5H-benzo[a]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)acetamide (15h)

Fig. 83: $^1$H NMR Spectrum of compound 15h
Brown solid; Reaction time 30 minutes (0.550 g, 97%); mp > 300 ºC; IR (KBr): 1105, 1148, 1298, 1317, 1364, 1400, 1514, 1588, 1628, 1703, 3301cm⁻¹; ¹H NMR (500 MHz, DMSO-d6) δ: 4.30 (s, 2H), 6.91 (d, 1H, J = 6.5 Hz), 6.98 (d, 2H, J = 8.5 Hz), 7.39 (t, 1H, J = 8.0 Hz), 7.44 (t, 1H, J = 8.0 Hz), 7.65 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 6.8 Hz), 7.84-7.88 (m, 4H), 7.91 (t, 1H, J = 7.5 Hz), 8.20 (d, 1H, J = 8.0 Hz), 8.68 (d, 2H, J = 8.2 Hz), 10.71 (s, 1H); ¹³C NMR (125 MHz, DMSO-d6) δ: 66.1, 102.1, 121.5, 124.5, 125.7, 126.5, 128.1, 128.8, 129.0, 129.7, 131.2, 133.0, 133.1, 133.3, 133.9, 135.3, 135.3, 136.9, 146.7, 165.9, 182.8; HRMS (EI) m/z: Calcd for C₃₀H₂₀ClN₃O₅S: 570.0149 Found: 570.0148.
3.5. Molecular docking studies

To understand the interaction of all the synthesized molecules (13, 14, 15a-h) with *Bacillus subtilis*, the crystal structure of ymaH from *Bacillus subtilis*\(^{30}\) (PDB ID: 3HSB) was downloaded from protein data bank and the molecular docking studies were performed using the GLIDE program\(^{31}\) (version 8.5, Schrodinger, LLC, New York, 2010). To analyze the docking results and execute the protocol, the maestro user interface (version 8.5, Schrodinger, LLC, New York, 2010) was employed and the validation of protocol was evaluated by redocking. The structures of compounds 13, 14, 15a-h were sketched using ACD/chemsketch (Freeware version). GLIDE grid generation wizard has been used to define the docking space. 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.\(^{32,33}\) The minimum inhibitory concentration (MIC) values were calculated by comparison with Sparfloxacin and Norfloxacin, which are the standard antibacterial drugs and they are presented in Table 6. All the cultures were prepared by Muller Hinton agar and the turbidity of all the antibacterial 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\(^5\) 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 \(\mu\)g/mL.\(^{34}\) 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 °C was done and the growth measured. The MIC of the tested compounds and the standard control drugs are tabulated in Table 6.

4. Results and discussion

4.1. Chemistry

The compound 2,3-dichloro-1,4-naphthoquinone was made to react with 4-aminophenyl sulfone to produce 6-(4-(4-aminophenylsulfonyl)phenylamino)-3-chloronaphthalene-1,4-dione (13). Previously compound 13 was prepared by Carroll et al., 1969\textsuperscript{24} following a difficult procedure. According to them, 2,3-dichloro-1,4-naphthoquinone reacts with hydrochloride salt of 4-amino phenyl sulfonamide in absolute ethanol medium and in the presence of \(N,N\)-diethyl aniline acting as the catalyst and the reaction was performed for 18 h under reflux condition. The overall percentage yield of compound 13 was reported to be only 72%. In our previous report we synthesized compound 13 by a simple and green route to achieved a yield of 88%.\textsuperscript{29} In this method, 2,3-dichloro-1,4-naphthoquinone was made to react with 4-aminophenyl sulfone (no hydrochloride salts of sulfonamides) in water as solvent and refluxing the mixture for 2 h. After cooling the reaction mixture at room temperature the precipitate was filtered by vacuum filtration and crystallized in 95% absolute ethanol. In comparison with the method of Carroll et al., our method had the advantage of giving better yield and also the preparative steps were green. The compound 14 was synthesized from compound 13, when it was reacted with an equal amount of 2-amino phenol in absolute ethanol medium under basic condition (equal amount of anhydrous \(K_2CO_3\)) and when the mixture was refluxed for 2 h. We attempted the same reaction in water medium but we were not successful because of the formation of a number of inseparable compounds. The compounds 15a-h was synthesized from compound 14,
when it was made to react with substituted acid chlorides in acetone medium without the aid of any base. In a previous report, the aminophenylsulfone is dissolved in pyridine and the substituted acid chloride is dissolved in dioxane and added drop wise to the reaction mixture and the reaction mixture was stirred overnight at room temperature. However in our reaction the compound reacts with substituted acid chlorides in acetone medium under reflux condition for duration of 30 minutes and the overall percentage yield obtained was between 95-98%.

Scheme 3: Synthesis of 2-(4-(4-aminophenylsulfonyl)phenylamino)-3-chloronaphthalene-1,4-dione (13), 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenoxazin-5-one (14) and N-(4-(4-(5-oxo-5H-benzo[a]phenoxazin-6-ylamino)phenylsulfonyl)phenyl)benzamides (15a-h).
This is the first report on the synthesis of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenoxazin-5-one derivatives (15a-h) to the best of our knowledge. Very few reports are available in literature about compounds with phenoxazin moiety. The molecular docking and \textit{in vitro} antibacterial studies have not been attempted on these systems so far.

4.2. Biology

4.2.1. Molecular docking studies of quinone derivatives

To understand the interaction of bacterial protein receptor with synthesized molecules (13, 14, 15a-h) the crystal structure of ymaH from \textit{Bacillus subtilis} was downloaded from protein data bank and studied with the glide program (See figures 85, 86). All the glide and E model scores are compared to the MIC of \textit{Bacillus subtilis} and the tested compounds are presented in table 5.

\textbf{Table 5:} Molecular docking studies of ten analogues taken for study with \textit{Bacillus subtilis} (BS) (PDB ID: 3HSB)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Glide score (kcal/mol)</th>
<th>E model score</th>
<th>MIC of BS (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>-4.656</td>
<td>-64.77</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>-3.972</td>
<td>-72.91</td>
<td>9</td>
</tr>
<tr>
<td>15a</td>
<td>-1.878</td>
<td>-85.61</td>
<td>11</td>
</tr>
<tr>
<td>15b</td>
<td>-1.410</td>
<td>-96.47</td>
<td>0.9</td>
</tr>
<tr>
<td>15c</td>
<td>-4.892</td>
<td>-87.94</td>
<td>8.2</td>
</tr>
<tr>
<td>15d</td>
<td>-5.364</td>
<td>-86.86</td>
<td>8.6</td>
</tr>
<tr>
<td>15e</td>
<td>-2.207</td>
<td>-103.48</td>
<td>31.6</td>
</tr>
<tr>
<td>15f</td>
<td>-5.170</td>
<td>-104.53</td>
<td>11.6</td>
</tr>
<tr>
<td>15g</td>
<td>-3.917</td>
<td>91.62</td>
<td>13</td>
</tr>
<tr>
<td>15h</td>
<td>-4.003</td>
<td>97.30</td>
<td>523</td>
</tr>
<tr>
<td>Sparfloxacina</td>
<td>-</td>
<td>-</td>
<td>9.76</td>
</tr>
<tr>
<td>Norfloxacina</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 \textit{Bacillus subtilis} (BS); aStandard antibacterial drugs.
Fig. 85: Docking model structures of compounds 15b into the ymaH binding pocket.

Fig. 86: Docking model structures of compounds 13 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 activity against two Gram-positive and five Gram-negative bacterias. All the compounds (13, 14, 15a-h) exhibited good antibacterial activity against Gram-negative bacteria of *Klebsiella pneumoniae* than the standard drugs used (Sparfloxacin and Norfloxacin). The compound 15b exhibits good activity against most of the Gram-positive and Gram-negative microorganisms due to the presence of the methyl group at the third position of the aromatic ring of the benzoyl unit. Compound 15b exhibits better activity against *Bacillus subtilis* (0.9 µg/mL), *Salmonella typhi* (376 µg/mL), *Pseudomonas aeruginosa* (22.5 µg/mL) and *Klebsiella pneumoniae* (357 µg/mL) than Sparfloxacin and Norfloxacin. Compound 3d (12 µg/mL) exhibits better activity against *Staphylococcus aureus* than the standard drug Norfloxacin (39.06 µg/mL). Compound 15c and 15d (523 µg/mL) exhibit better antibacterial activity against *Escherichia coli* than Norfloxacin (625 µg/mL). Compound 15e (0.7 µg/mL) exhibits better and good activity among all the molecules synthesized against *Proteus vulgaris* than Sparfloxacin (4.8 µg/mL). Standard drug Norfloxacin did not exhibit any activity against *Bacillus subtilis* and *Proteus vulgaris* microorganisms. Compound 13 did not exhibit any inhibition against *Proteus vulgaris*, *Salmonella typhi* and compound 15g did not exhibit any inhibition against *Staphylococcus aureus* (See table 6).

In connection with the structure–activity relationship of the synthesized molecules (13, 14, 15a–h), compounds having electronegative atoms or having both electron withdrawing and electron donating functional groups (chloro, nitro and methyl groups) exhibit better *in vitro* antibacterial activity. The steric effect does not play any role to affect or enhance the biological activities. Moreover, from the observation of *in vitro* antibacterial study of synthesized molecules, the zinc binding region (ZBR) and the phenoxazin moiety play an important role. The compounds without ZBR and the phenoxazin moiety exhibit low antibacterial activity (Compounds 13 and 14).
Pharmacophoric features of the title compounds (15a-n).

**Table 6: In vitro antibacterial activity of phenoxazin-5-one derivatives against Gram-positive and Gram-negative bacteria (MIC in µg/mL)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>B. subtilis (Clinical Isolated)</th>
<th>S. aureus (Clinical Isolated)</th>
<th>E. coli (Clinical Isolated)</th>
<th>P. vulgaris (Clinical Isolated)</th>
<th>S. typhi (Clinical Isolated)</th>
<th>P. aeruginosa (Clinical Isolated)</th>
<th>K. pneumoniae (Clinical Isolated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>1.4</td>
<td>34</td>
<td>762</td>
<td>*</td>
<td>*</td>
<td>99.2</td>
<td>1015</td>
</tr>
<tr>
<td>14</td>
<td>9</td>
<td>11.5</td>
<td>532</td>
<td>134</td>
<td>245</td>
<td>48.5</td>
<td>825</td>
</tr>
<tr>
<td>15a</td>
<td>11</td>
<td>14</td>
<td>634</td>
<td>104</td>
<td>243</td>
<td>29.3</td>
<td>783</td>
</tr>
<tr>
<td>15b</td>
<td><strong>0.9</strong></td>
<td>*</td>
<td>621</td>
<td>97</td>
<td>376</td>
<td>22.5</td>
<td><strong>357</strong></td>
</tr>
<tr>
<td>15c</td>
<td>8.2</td>
<td>29</td>
<td><strong>523</strong></td>
<td>139</td>
<td>523</td>
<td>101.5</td>
<td>897</td>
</tr>
<tr>
<td>15d</td>
<td>8.6</td>
<td>12</td>
<td><strong>523</strong></td>
<td>107</td>
<td>254</td>
<td>32.5</td>
<td>657</td>
</tr>
<tr>
<td>15e</td>
<td>31.6</td>
<td>29</td>
<td>621</td>
<td><strong>0.7</strong></td>
<td>329</td>
<td>124</td>
<td>567</td>
</tr>
<tr>
<td>15f</td>
<td>31.6</td>
<td>19</td>
<td>582</td>
<td>83</td>
<td>523</td>
<td><strong>14</strong></td>
<td>653</td>
</tr>
<tr>
<td>15g</td>
<td>13</td>
<td>*</td>
<td>573</td>
<td>127</td>
<td>245</td>
<td>98</td>
<td>896</td>
</tr>
<tr>
<td>15h</td>
<td>523</td>
<td>27</td>
<td>638</td>
<td>57</td>
<td>678</td>
<td>117</td>
<td>725</td>
</tr>
<tr>
<td>Sparfloxacina</td>
<td>9.76</td>
<td>4.87</td>
<td>156.3</td>
<td>4.8</td>
<td>2500</td>
<td>156.3</td>
<td>2500</td>
</tr>
<tr>
<td>Norfloxacina</td>
<td>*</td>
<td>39.06</td>
<td>625</td>
<td>*</td>
<td>627</td>
<td>39.06</td>
<td>&lt;1.2</td>
</tr>
</tbody>
</table>

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

A new series of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenoxazin-5-one derivatives were synthesized and characterized by FT-IR, $^1$H NMR, $^{13}$C NMR and high resolution mass (HRMS-EI) spectral analyses. All the molecules were studied for their interactions with Bacillus subtilis by molecular docking protocol. In vitro antibacterial activity of the tested compounds shows improved activity against all the microorganisms used. In particular compound 15d exhibits marked activity against four microorganisms. Compound 15e (0.7 µg/mL) exhibited good activity against Proteus vulgaris than the standard drug Sparfloxacin (4.8 µg/mL) used.
References


