5. Chemistry

5.1. 2-((3-(4-substituted-piperazin-1-yl)-3-oxopropyl)thio)naphthalene-1,4-Dione (Series I)

Based on the comprehensive description of the structural and functional aspect, 1, 4-naphthoquinone analogues have been designed, synthesized and evaluated for their anticancer activity. 1,4-Naphthoquinone derivatives with various substituted piperazines (**MB-1** to **MB-19**, **series I)** and substituted oxadiazoles **(MB-20** to **MB-33, series II)** has been synthesized (Tandon *et al.,* 2004) depicted in **Figure 4.1** and **Figure 4.2**. The reaction process was optimized by varying the stirring time, solvents, temperature and monitored using thin-layer chromatography (TLC).

5.2. Characterization of Piperazine Substituted 1, 4-Naphthoquinone

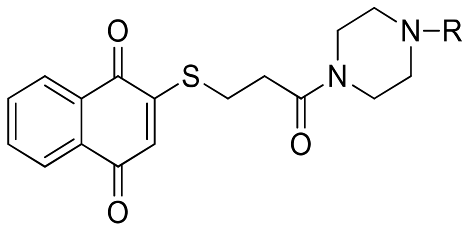
The Fourier transform infrared (FT-IR) spectrum of compounds **MB-1** to **MB-19** demonstrate a strong band at ~3400-3200 cm^{-1} corresponding to the N-H stretching vibrations. The aromatic nucleus C-H stretching in the structural skeleton of these compounds was observed in the range of 3100-3000 cm^{-1} of C-H stretching bands. The C=O stretching of amide groups of entitled compounds was observed at 1698 cm^{-1} - 1739 cm^{-1}. The existence of the carboxylic acid group in the newly prepared compounds was established by the disappearance of -OH band (carboxylic acid), at 3358.31 cm^{-1} in the FT-IR spectra and occurrence of a characteristic band of the carbonyl group. The disappearance of OH proton at δ 9.47 ppm in ^{1}H NMR spectra of the synthesized compounds was established by the substitution at the hydroxyl group. However the aromatic protons of the synthesized compound resonated from δ 8.37 to 6.15 ppm in ^{1}H NMR spectra because of anisotropic effect of the ring. In synthesized compounds **MB-1 to MB-19**, the characteristic peak of methylene group was appeared in the range δ 2.00-3.00 ppm. Compound **MB-6** showed a singlet at δ 2.20 of the methyl group. Two singlet was observed at δ 2.38 and 2.29 ppm for two methyl groups in compound **MB-17**. Singlet for methoxy protons were appeared at their usual position δ 3.80 **(MB-18)**.

In the ^{13}C NMR spectrum of the target compounds, the carbonyl carbon was observed at ≃ δ 182.0 ppm and 176.0 of naphthalene ring and aliphatic carbonyl carbon was at ≃ 170.0 ppm and aromatic carbons was observed in the range from δ 155.00-102.00 ppm in the synthesized compound. However the methylene carbon for compounds **MB-1** to **MB-19** was emerged at δ 16.19-8.59 ppm.

The ESI-MS spectrum of MB- 7 [453.80 (M^+), 455.810 ($M^+ + 2$)], MB-8 [488.53 (M^+), 490.53 ($M^+ + 2$)] and MB-9 [454.81 (M^+), 456.81 ($M^+ + 2$)] showed a molecular ion peak [M^+] at and approximately one third intensity $[M+2]^+$ peak due to isotopic abundance of ^{17}Cl. The CHN elemental analyses supported the proposed structures and ascertained the purity of the newly synthesized analogues.

Piperazine substituted 1, 4-naphthoquinone derivatives and their physiochemical chemical properties are mentioned in **Table 5.1**.

Table 5.1: Chemical structure of various piperazines substituted 1, 4-naphthoquinone derivatives **(MB-1** to **MB-19)**



(MB-1 to **MB-19)**

Compounds	R	Molecular weight	Melting point (°C)	Appearance	Log P	% Yield	R_f
MB-1	H	330.40	155-157	Yellow	-0.41	58	0.49
MB-2	C_6H_5-	405.71	202-204	White	2.04	63	0.53
MB-3	$(C_6H_5)_2CH_2$-	495.67	189-191	Yellow	3.41	64	0.68
MB-4	$C_6H_5CH_2$-	420.34	162-164	Brownish	1.70	71	0.51
MB-5	C_5H_4N-	407.43	172-174	Brownish	1.42	61	0.50
MB-6	2-$CH_3C_6H_5$-	420.84	210-212	Creamy	2.53	69	0.56
MB-7	2-ClC_6H_5-	440.23	223-225	Creamy	2.60	68	0.58
MB-8	2,3-ClC_6H_5-	474.45	237-239	White	3.16	73	0.62
MB-9	4-ClC_6H_5-	440.12	192-194	Brownish	2.60	66	0.58

MB-10	2-FC_6H_5-	424.57	179-181	Creamy	2.20	70	0.55
MB-11	4-FC_6H_5-	424.13	198-200	Yellow	2.55	68	0.57
MB-12	4-$CF_3C_6H_5$-	474.12	214-116	Yellow	3.31	69	0.64
MB-13	3-FC_6H_5-	424.13	168-170	White	2.55	66	0.57
MB-14	4-$OCH_3C_6H_5$-	436.15	143-145	White	2.27	69	0.55
MB-15	2-$OCH_3C_6H_5$-	436.15	151-153	Brownish	2.27	65	0.55
MB-16	4-$NO_2C_6H_5$-	451.12	240-242	White	2.53	63	0.56
MB-17	2,3-$(CH_3)_2C_6H_5$-	434.17	229-231	White	3.37	70	0.66
MB-18	2,3-$(OCH_3)_2$-4-BrC_6H_5-	560.08	146-148	Yellow	2.63	68	0.59
MB-19	2-$NO_2C_6H_5$-	451.49	260-262	Creamy	2.53	67	0.56

5.3. Spectral Characterization

3-((3-methyl-1, 4-dioxo-1, 4-dihydro naphthalen-2-yl) thio) propanoic acid (3)

FT-IR (KBr υ cm^{-1}): 3337.90 (O-H), 3110.83 (aromatic C-H stretch), 2953.84 (aliphatic C-H stretch), 1701.72 (C=O stretch), 1656.70 (C=O stretch), 1547.63 (C=C ring stretch), 1235.42 (C-O stretch); ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm, δ): 9.47 (s, 1H, COO*H*), 8.23 (m, 2H, Ar*H*), 7.70 (m, 2H, Ar*H*), 3.47 (t, 2H, SCH_2, *J* = 6.60 Hz), 2.70 (t, 2H, CH_2C=O, J = 6.60 Hz), 2.45 (s, 3H, CH_3).

2-Methyl-3-((3-oxo-3-(piperazin-1-yl) propyl) thio) naphthalene-1, 4-dione (MB-1)

FT-IR (KBr υ cm^{-1}): 3320.12 (N-H stretch), 3068.08 (aromatic C-H stretch), 2927.11 (aliphatic C-H stretch), 1735.77 (C=O stretch, 1689.24 (C=O stretch), 1624.70, 1505.83 (C=C ring stretch), 1268.44, 1215.39 (C-O stretch),734.38 (C-H bend); ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm): δ 8.16 (m, 2H, Ar*H*), 7.72 (m, 2H, Ar*H*), 3.56 (t, 2H, *J*= 4.12 Hz, -N-CH_2), 3.33 (m, 4H, -N-CH_2; -SCH_2), 2.88 (m, 6H, NH$(CH_2)_2$; -CH_2CO), 2.77 (s, 3H, CH_3) and 1.27 (s, 1H, -N*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.73, 178.54, 171.15, 139.11, 137.48, 133.84, 132.95, 130.94, 128.10, 46.84, 44.92, 35.73, 29.56, 12.40; ESI-MS: *m/z* 510.55; Anal. $C_{31}H_{30}N_2O_3S$: C, 72.37; H, 5.10; N, 5.21; Found: C, 72.56; H, 5.21; N, 5.27.

2-Methyl-3-((3-oxo-3-(4-phenylpiperazin-1-yl) propyl) thio) naphthalene-1,4-dione (MB-2)

FT-IR (KBr υ cm^{-1}): 3068.08 (aromatic C-H stretch); 2916.47 (aliphatic C-H stretch), 1738.78 (C=O stretch), 1690.04 (C=O stretch), 1623.42 (C=C ring stretch), 1227.74 (C-O stretch) and 750.23 cm^{-1} (C-H bend). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm δ): 8.25 (dd, 1H, J_m = 1.44 Hz, J_o = 5.60 Hz, Ar*H*), 8.17 (dd, 1H, J_m = 1.40 Hz, J_o = 5.80 Hz, Ar*H*), 7.74 (m, 2H, Ar*H*), 7.07 (t, 2H, *J* = 5.88 Hz, Ar*H*), 6.62 (m, 3H, Ar*H*), 3.79 (m, 4H, -N$(CH_2)_2$), 3.66 (m, 2H, N-CH_2), 3.46 (m, 2H, N-CH_2), 3.13 (t, 2H, *J* = 4.36 Hz, -

SCH_2), 3.00 (t, 2H, J = 4.40 Hz, -CH_2CO) and 2.76 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.73, 178.54, 171.40, 150.96, 139.68, 137.51, 133.84, 132.92, 130.94, 129.35, 128.15, 120.31, 116.77, 49.17, 44.84, 35.73, 29.56, 15.12; ESI-MS: m/z: 419.65 Anal. $C_{24}H_{24}N_2O_3S$: C, 68.32; H, 5.46; N, 6.39; Found: C, 68.42; H, 5.36; N, 6.34.

2-((3-(4-benzhydrylpiperazin-1-yl)-3-oxopropyl)thio)-3-methylnaphthalene-1,4-dione (MB-3)

FT-IR (KBr υ cm^{-1}): 3384.66 (aromatic C-H stretch), 2943.91 (aliphatic C-H stretch), 1724.39 (C=O stretch), 1695.18 (C=O stretch), 1629.13, 1585.27 (C=C ring stretch), 1218.84 (C-O stretch), 733.20cm^{-1} (C-H bend); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 8.04 (dd, 1H, J_m = 1.40 Hz, J_o = 5.80 Hz ArH), 8.00 (dd, 1H, J_m = 1.40 Hz, J_o = 5.80 Hz, ArH), 7.69 (m, 2H, ArH), 7.27 (m, 11H, ArH), 3.52 (t, 2H, J = 4.08 Hz, -N-CH_2), 3.42 (t, 2H, J = 4.08 Hz, -N-CH_2), 3.34 (t, 2H, J = 4.16 Hz, -SCH_2), 2.96 (t, 2H, J = 4.08 Hz, -N-CH_2), 2.90 (t, 2H, J = 4.08, -N-CH_2), 2.64 (m, 5H, -CH_2CO; CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 184.55, 179.34, 171.80, 140.56, 139.46, 137.51, 133.84, 132.40, 130.94, 129.28, 128.85, 128.39, 126.73, 77.58, 50.80, 44.55, 35.73, 29.56, 13.12; ESI-MS: m/z 510.55; Anal. $C_{31}H_{30}N_2O_3S$: C, 72.37; H, 5.10; N, 5.21; Found: C, 72.56; H, 5.21; N, 5.27.

2-((3-(4-benzylpiperazin-1-yl)-3-oxopropyl)thio)-3-methylnaphthalene-1,4-dione (MB-4)

FT-IR (KBr υ cm^{-1}): 3035.42 (aromatic C-H stretch), 2925.02 (aliphatic C-H stretch), 1735.81 (C=O stretch),1692.80 (C=O stretch), 1627.99 (C=C ring stretch), 1233.26 (C-O stretch), 736.29 (C-H bend); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 8.18 (dd, 1H, J_m = 1.41 Hz, J_o = 5.80 Hz, ArH), 8.05 (dd, 1H, J_m = 1.40 Hz, J_o = 5.82 Hz, ArH), 7.75 (m, 2H, ArH), 7.25 (m, 5H, ArH), 3.62 (m, 4H, -NH(CH_2)$_2$), 3.44 (t, 2H, J = 6.66 Hz, -N-CH_2-

C), 3.35 (t, 2H, J = 4.24 Hz, -N-CH_2), 2.88 (m, 4H, -SCH_2; -CH_2CO), 2.66 (t, 2H, J = 4.24 Hz, N-CH_2) and 2.48 (s, 3H,CH_3); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 186.55 , 178.54, 177.95, 139.46, 138.39, 137.17, 133.84, 132.38, 130.33, 128.93, 127.90, 127.90, 126.55, 63.66, 51.15, 44.25, 35.73, 29.82, 4.25; ESI-MS: *m/z* 434.31; Anal. $C_{25}H_{26}N_2O_3S$: C, 69.56; H, 6.21; N, 6.94; Found: C, 69.34; H, 6.32; N, 6.51.

2-Methyl-3-((3-oxo-3-(4-(pyridin-2-yl) piperazin-1-yl) propyl) thio) naphthalene-1,4-dione (MB-5)

FT-IR (KBr, υ cm^{-1}): 3039.73 (aromatic C-H stretch), 2923.04 (aliphatic C-H stretch), 1737.11 (C=O stretch), 1690.13 (C=O stretch), 1627.77, 1584.63 (C=C ring stretch), 1271.80 (C-O stretch), 734.04 cm^{-1} (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.13 (dd, 1H, J_m = 1.16 Hz, J_o = 6.00 Hz Ar*H*), 8.03 (m, 2H, Ar*H*), 7.68 (m, 2H, Ar*H*), 7.51 (m, 1H, Ar*H*), 6.67 (m, 2H, Ar*H*), 3.74 (m, 2H, -SCH_2), 3.64 (m, 4H, -N(CH_2)$_2$), 3.51 (m, 2H, -CH_2CO), 3.30 (t, 2H, J = 6.48 Hz, -N-CH_2), 2.90 (t, 2H, J = 6.48, -N-CH_2) and 2.37 (s, 3H, CH_3); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 184.84, 179.46, 170.94, 158.90, 148.87, 140.12, 139.17, 137.21, 133.02, 132.17, 128.91, 127.62, 113.61, 107.70, 47.24, 44.32, 35.15, 29.95,13.84; ESI-MS: *m/z* 421.97; Anal. $C_{23}H_{23}N_3O_3S$: C, 65.92; H, 5.53; N, 9.23; Found: C, 65.50; H, 5.82; N, 9.14.

2-Methyl-3-((3-oxo-3-(4-(o-tolyl) piperazin-1-yl) propyl) thio) naphthalene-1,4-dione (MB-6)

FT-IR (KBr, υ cm^{-1}): 3030.29 (aromatic C-H stretch), 2918.25 (aliphatic C-H stretch), 1732.48 (C=O stretch), 1694.49 (C=O stretch), 1625.11 (C=C ring stretch), 1272.33 (C-O stretch), 776.76 cm^{-1} (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.22 (dd, 1H, J_m = 1.36 Hz, J_o = 5.84 Hz, Ar*H*), 8.11 (dd, 1H, J_m = 1.36 Hz, J_o = 5.84 Hz, Ar*H*), 7.66 (m, 2H, Ar*H*), 6.91 (m, 2H, Ar*H*), 6.55 (m, 2H, Ar*H*), 3.75 (dt, 4H, J_m = 3.96 Hz, J_o = 16.92 Hz, -N(CH$_2$)$_2$), 3.53 (t, 2H, J = 3.96 Hz, -S-CH_2), 3.40 (t, 2H,J = 3.92 Hz, -

C*H*$_2$CO), 3.23 (t, 2H, *J* = 4.48 Hz, -N-C*H*$_2$), 2.95 (t, 2H, *J* = 4.48 Hz, -N-C*H*$_2$), 2.72 (s, 3H, C*H*$_3$), 2.20 (s, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 188.15, 174.84, 173.84, 150.93, 139.27, 133.50, 132.47, 130.54, 130.06, 129.40, 128.30, 127.99, 127.10, 120.99, 117.82, 50.27, 44.14, 35.73, 29.36, 17.23,13.73; ESI-MS: *m/z* 433.12; Anal. $C_{25}H_{26}N_2O_3S$: C, 69.20; H, 6.03; N, 7.07; Found: C, 69.32; H, 6.18; N, 6.98.

2-((3-(4-(2-chlorophenyl) piperazin-1-yl)-3-oxopropyl) thio)-3-methylnaphthalene-1,4-dione (MB-7)

FT-IR (KBr, υ cm^{-1}): 3057.01 (aromatic C-H stretch), 2916.26 (aliphatic C-H stretch), 1738.63 (C=O stretch), 1690.14 (C=O stretch), 1625.26 (C=C ring stretch), 1226.67 (C-O stretch), 747.73 (C-H bend), 675.74 (C-Cl Stretch); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.08 (m, 2H, Ar*H*), 7.74 (m, 2H, Ar*H*), 7.09 (m, 1H, Ar*H*), 6.96 (td, 1H, *J* = 4.42 Hz, Ar*H*), 6.61 (m, 2H, Ar*H*), 3.82 (t, 2H, *J* = 4.08 Hz, N-C*H*$_2$), 3.68 (t, 2H, *J* = 4.08 Hz, N-C*H*$_2$), 3.53 (t, 2H, *J* = 4.08 Hz, -N-C*H*$_2$), 3.44 (m, 4H, -N-C*H*$_2$; -SC*H*$_2$), 3.02 (t, 2H, *J* = 5.12 Hz, -C*H*$_2$CO), 2.28 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 187.18, 175.73, 170.79, 148.43, 139.92, 137.66, 133.84, 132.35, 131.75, 130.51, 128.33, 127.62, 127.27, 120.56, 119.18, 50.40, 44.27, 35.47, 29.01, 16.12 (*C*H$_3$); ESI-MS: *m/z* 453.80 (M$^+$), 455.810 (M$^+$ + 2); Anal. $C_{24}H_{23}Cl\ N_2O_3S$: C, 63.32; H, 5.20; N, 6.98; Found: C, 63.42; H, 5.13; N, 6.84.

2-((3-(4-(2,3-dichlorophenyl)piperazin-1-yl)-3-oxopropyl)thio)-3-methylnaphthalene-1,4-dione (MB-8)

FT-IR (KBr, υ cm^{-1}): 3038.75 (aromatic C-H stretch), 2926.93 (aliphatic C-H stretch), 1738.68 (C=O stretch), 1684.51 (C=O stretch), 1627.31, 1500.84 (C=C ring stretch), 1251.13 (C-O stretch), 808.65 (C-H bend), 708.43 (C-Cl Stretch); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.02 (m, 2H, Ar*H*), 7.66 (m, 2H, Ar*H*), 6.88 (t, 1H, *J* = 5.96, Ar*H*), 6.63 (dd, 1H, *J* = 4.13 Hz, Ar*H*), 6.55 (m, 1H, Ar*H*), 3.80 (t, 2H, *J* = 4.08 Hz,N-C*H*$_2$), 3.74

(t, 2H, *J* = 4.12 Hz, N-C*H*$_2$), 3.65 (t, 2H, *J* = 4.08 Hz, -N-C*H*$_2$), 3.41 (m, 4H, -N-C*H*$_2$; -SC*H*$_2$), 2.98 (t, 2H, *J* = 4.32 Hz, -C*H*$_2$CO), 2.43 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.12, 178.94, 171.46, 149.83, 139.39, 137.93, 134.73, 133.95, 132.09, 130.73, 128.56, 128.15, 127.84, 121.12, 118.47, 50.22, 44.97, 35.32, 29.38, 12.41; ESI-MS: *m/z*: 488.53 (M$^+$), 490.53 (M$^+$ + 2); Anal. $C_{24}H_{22}Cl_2$ N_2O_3S: C, 58.86; H, 4.32; N, 6.00; Found: C, 58.57; H, 4.40; N, 5.92.

2-((3-(4-(4-chlorophenyl) piperazin-1-yl)-3-oxopropyl) thio)-3-methylnaphthalene-1,4-dione (MB-9)

FT-IR (KBr, υ cm^{-1}): 3073.30 (aromatic C-H stretch), 2928.41 (aliphatic C-H stretch), 1728.47 (C=O stretch), 1685.30 (C=O stretch), 1629.75, 1592.24 (C=C ring stretch), 1453.39 (C-N stretch), 1244.41 (C-O stretch), 757.93 (C-H bend), 687.20; ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.06 (m, 2H, Ar*H*), 7.71 (m, 2H, Ar*H*), 7.12 (d, 2H, *J* = 6.00 Hz, Ar*H*), 6.61 (d, 2H, *J* = 5.96 Hz, Ar*H*), 3.77 (m, 4H, N(C*H*$_2$)$_2$), 3.62 (m, 2H, -SC*H*$_2$), 3.45 (m, 4H, -N(C*H*$_2$)$_2$), 2.98 (t, 2H, *J* = 6.44 Hz, -C*H*$_2$CO), 2.56 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 186.98, 173.84, 165.38, 150.29, 139.23, 137.40, 133.84, 132.58, 132.58, 130.44, 129.98, 128.33, 127.90, 117.28, 49.39, 44.61, 35.65, 29.21, 8.25; ESI-MS: *m/z* 454.81 (M$^+$) 456.81 (M$^+$ + 2); Anal. $C_{24}H_{23}Cl$ N_2O_3S: C, 63.76; H, 5.12; N, 6.88; Found: C, 63.52; H, 5.22; N, 6.80.

2-((3-(4-(2-fluorophenyl) piperazin-1-yl)-3-oxopropyl) thio)-3-methylnaphthalene-1,4-dione (MB-10)

FT-IR (KBr, υ cm^{-1}): 3053.48 (aromatic C-H stretch), 2950.20 (aliphatic C-H stretch), 1740.99 (C=O stretch), 1680.05 (C=O stretch), 1621.85, 1584.62 (C=C ring stretch), 1217.26 (C-O stretch), 1120.82 (C-F Stretch), 766.20 (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.17 (m, 2H, Ar*H*), 7.70 (m, 2H, Ar*H*), 6.79 (m, 2H, Ar*H*), 6.56 (m, 2H, Ar*H*), 3.80 (t, 2H, *J* = 3.98 Hz, N-C*H*$_2$), 3.71 (t, 2H, *J* = 3.98 Hz, N-C*H*$_2$), 3.57 (t, 2H, *J*

= 4.00 Hz, -N-CH_2), 3.41 (t, 2H, *J* = 3.98 Hz, -NCH_2), 3.35 (t, 2H, *J* = 5.34 Hz, -S-CH_2), 2.88 (t, 2H, *J* = 6.46 Hz, -CH_2CO), 2.74 (s, 3H, CH_3); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.04, 179.46, 172.40, 154.75, 142.68, 139.19, 137.96, 135.27, 133.67, 132.15, 127.86, 125.88, 119.76, 118.31, 117.17, 50.75, 44.35, 35.31, 29.66, 17.03; ESI-MS: *m/z* 437.94 (M$^+$); 439.94 (M$^+$+ 2); Anal. $C_{24}H_{23}FN_2O_3S$: C, 66.12; H, 5.84; N, 6.10; Found: C, 65.99; H, 5.63; N, 6.24.

2-((3-(4-(4-fluorophenyl)piperazin-1-yl)-3-oxopropyl)thio)-3-methylnaphthalene-1,4-dione (MB-11)

FT-IR (KBr, υ cm^{-1}): 3066.96 (aromatic C-H stretch), 2938.45 (aliphatic C-H stretch), 1737.96 (C=O stretch), 1695.47 (C=O stretch), 1625.73,16001.61 (C=C ring stretch), 1217.37 (C-O stretch),1087.15 (C-F Stretch), 754.43 (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.14 (m, 2H, Ar*H*), 7.67 (m, 2H, Ar*H*), 6.76 (t, 2H, *J* = 6.10 Hz, Ar*H*), 6.55 (m, 2H, Ar*H*), 3.75 (m, 4H, N(CH_2)$_2$), 3.53 (t, 2H, *J* = 3.88 Hz, -N-CH_2), 3.41 (t, 2H, *J* = 3.88 Hz, -N-CH_2), 3.26 (t, 2H, *J* = 4.46, -SCH_2), 2.97 (m, 5H,-CH_2CO; CH_3); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 184.59, 176.98, 172.40, 158.25, 149.49, 139.17, 137.66, 133.77, 132.35, 130.93, 127.37, 118.59, 117.68, 49.95, 44.33, 35.38, 29.59, 14.84; ESI-MS: *m/z* 438.75 (M$^+$), 440.75 (M$^+$ + 2); Anal. $C_{24}H_{23}FN_2O_3S$: C, 65.54; H, 5.76; N, 6.91;Found: C, 65.83; H, 5.89; N, 6.36.

2-Methyl-3-((3-oxo-3-(4-(4-(trifluoromethyl) phenyl) piperazin-1yl) propyl) thio) naphthalene-1,4-dione (MB-12)

FT-IR (KBr, υ cm^{-1}): 3079.04 (aromatic C-H stretch), 2927.33 (aliphatic C-H stretch), 1721.44 (C=O stretch), 1657.60 (C=O stretch), 1625.03, 1600.35 (C=C ring stretch), 1444.63 (C-N stretch), 1248.82 (C-O stretch), 1125.93 (C-F stretch), 773.40 cm^{-1} (C-H bend). ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.20 (m, 2H, Ar*H*), 7.72 (m, 2H, Ar*H*), 7.33 (d, 2H, *J* = 5.96 Hz, Ar*H*), 6.60 (d, 2H, *J* = 5.96 Hz, Ar*H*), 3.78 (m, 4H, N(CH_2)$_2$), 3.64

(m, 2H, -N-C*H*$_2$), 3.47 (m, 2H, -N-C*H*$_2$), 3.31 (t, 2H, *J* = 4.46 Hz, -SC*H*$_2$) and 3.02 (m, 5H,-C*H*$_2$CO; C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz δ): 184.24, 175.12, 173.84, 154.24, 139.25, 137.49, 133.17, 132.77, 130.85, 128.00, 127.51, 126.85, 124.46, 114.17, 49.96, 44.38, 35.81, 29.81, 14.24; ESI-MS: *m/z* 488.06 (M$^+$), 490.06 (M$^+$ + 2); Anal. $C_{25}H_{23}F_3N_2O_3S$: C, 61.40; H, 4.25; N, 5.55; Found: C, 61.22; H, 4.45; N, 5.76.

2-((3-(4-(3-fluorophenyl) piperazin-1-yl)-3-oxopropyl) thio)-3-methylnaphthalene-1,4-dione (MB-13)

FT-IR (KBr, υ cm^{-1}): 3038.75 (aromatic C-H stretch), 2926.93 (aliphatic C-H stretch), 1738.68 (C=O stretch), 1684.91 (C=O stretch), 1627.31, 1591.91 (C=C ring stretch), 1251.13 (C-O stretch), 1087.76 (C-F stretch), 708.43 (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.32 (dd, 1H, J_m = 1.52 Hz, J_o = 5.60 Hz, Ar*H*), 8.17 (dd, 1H, J_m = 1.52 Hz, J_o = 5.60 Hz, Ar*H*), 7.72 (m, 2H, Ar*H*), 7.06 (m, 1*H*, Ar*H*), 6.41 (m, 3H, Ar*H*), 3.88 (m, 2H, -N-C*H*$_2$), 3.72 (m, 4H, -N(C*H*$_2$)$_2$), 3.46 (m, 2H, -N-C*H*$_2$), 3.36(t, 2H, *J* = 4.56 Hz, -SC*H*$_2$), 3.01 (t, 2H, *J* = 4.56 Hz, -C*H*$_2$CO), 2.80 (s, 3H,C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 183.48, 179.46, 170.94, 163.20, 139.318, 137.73, 133.64, 133.11, 132.40, 130.29, 128.97, 127.90, 113.76, 107.73, 103.48, 49.80, 44.30, 35.28, 29.27, 15.56; ESI-MS: *m/z* 438.32 (M$^+$), 440.32 (M$^+$ + 2); Anal. $C_{24}H_{23}FN_2O_3S$: C, 65.76; H, 5.35; N, 6.30; Found: C, 65.88; H, 5.47; N, 6.21.

2-((3-(4-(4-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)thio)-3-methyl naphthalene-1,4-dione (MB-14)

FT-IR (KBr υ cm^{-1}): 3073.30 (aromatic C-H stretch), 2928.41 (aliphatic C-H stretch), 1728.47 (C=O stretch), 1685.30 (C=O stretch),1629.75, 1592.24 (C=C ring stretch), 1244.41 (C-O stretch), 757.93 (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz δ): 8.11 (m, 2H, Ar*H*), 7.73 (m, 2H, Ar*H*), 6.72 (d, 2H, *J* = 6.04 Hz, Ar*H*), 6.66 (d, 2H, *J* = 6.04 Hz, Ar*H*), 3.82 (m, 5H, OC*H*$_3$; -N-C*H*$_2$), 3.69 (m, 4H, N(C*H*$_2$)$_2$), 3.44 (m, 4H, -N-C*H*$_2$; -

SCH_2), 2.98 (t, 2H, J = 4.14 Hz, -CH_2CO), 2.70 (s, 3H,CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 186.20, 176.19, 173.84, 152.81, 143.73, 139.17, 137.58, 133.82, 132.81, 130. 47, 127.90, 116.20, 116.19, 56.04, 49.51, 44.76, 35.16, 29.16, 16.19; ESI-MS: *m/z* 450.41; Anal. $C_{25}H_{26}N_2O_4S$: C, 66.31; H, 5.90; N, 6.21; Found: C, 66.87; H, 5.63; N, 6.39.

2-((3-(4-(2-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)thio)-3methyl naphthalene - 1,4-dione (MB-15)

FT-IR (KBr υ cm^{-1}): 3053.48 (aromatic C-H stretch), 2950.20 (aliphatic C-H stretch), 1740.99 (C=O stretch), 1680.05 (C=O stretch), 1621.85 (C=C ring stretch), 1217.26 (C-O stretch), 812.78; ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 8.07 (m, 2H, Ar*H*), 7.72 (m, 2H, Ar*H*), 6.84 (m, 1H, Ar*H*), 6.77 (m, 1H, Ar*H*), 6.71 (m, 2H, Ar*H*), 3.83 (t, 2H, J = 3.90 Hz, -N-CH_2), 3.78 (s, 3H, OCH_3), 3.74 (t, 2H, J = 3.88 Hz, -N-CH_2), 3.68 (t, 2H, J = 3.88 Hz, -N-CH_2), 3.49 (t, 2H, J = 3.88 Hz, -N-CH_2), 3.40 (t, 2H, J = 6.46 Hz, -SCH_2), 2.99 (t, 2H, J = 6.46 Hz, -CH_2CO), 2.60 (s, 3H, CH_3),; ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.84, 179.46, 172.64, 149.31, 142.64, 139.50, 133.37, 132.32, 130.30, 128.29, 122.28, 120.27, 117.27, 113.15, 56.79, 50.54, 44.55, 35.75, 29.73,15.84; ESI-MS: *m/z* 450.27; Anal. $C_{25}H_{26}N_2O_4S$: C, 66.87; H, 5.43; N, 6.25; Found: C, 66.36; H, 5.49; N, 6.35.

2-Methyl-3-((3-(4-(4-nitrophenyl) piperazin-1-yl)-3-oxopropyl) thio) naphthalene-1,4-dione (MB-16)

FT-IR (KBr υ cm^{-1}): 3062.97 (aromatic C-H stretch), 2909.20 (aliphatic C-H stretch), 1728.33 (C=O stretch),1703.88 (C=O stretch), 1630.86, 1602.9 (C=C ring stretch), 1450.99 (C-N stretch), 1266.14 (C-O stretch), 746.78 (C-H bend); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06 (m, 4H, Ar*H*), 7.72 (m, 2H, Ar*H*), 6.91 (d, 2H, J = 5.92 Hz, Ar*H*), 3.83 (m, 2H, -N-CH_2), 3.77 (m, 4H, -N(CH_2)$_2$), 3.55 (m, 2H, -N-CH_2), 3.42 (t, 2H, J =

6.46 Hz, -SC*H*$_2$), 2.98 (t, 2H, *J* = 6.50 Hz, -C*H*$_2$CO), 2.63 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.94, 174.84, 171.81, 158.30, 139.44, 137.88, 133.82, 132.40, 130.40, 128.33, 127.90, 126.40, 112.41, 49.44, 44.95, 35.31, 29.77, 15.23; ESI-MS: *m/z* 465.60; Anal.

$C_{24}H_{23}N_3O_5S$: C, 61.80; H, 4.65; N, 9.28; Found: C, 61.88; H, 4.83; N, 9.19.

2-((3-(4-(2,3-dimethylphenyl)piperazin-1-yl)-3-oxopropyl)thio)-3-methyl naphthalene-1,4-dione (MB-17)

FT-IR (KBr, υ cm^{-1}): 3079.32 (aromatic C-H stretch), 2926.70 (aliphatic C-H stretch), 1720.40 (C=O stretch), 1684.73 (C=O stretch), 1592.24 (C=C ring stretch), 1251.73 (C-O stretch), 707.61; ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.28 (m, 1H, Ar*H*), 8.17 (m, 1H, Ar*H*), 7.83 (m, 2H, Ar*H*), 6.95 (t, 1H, *J* = 6.46 Hz, Ar*H*), 6.57 (dd, 1H *J* = 5.32 Hz, Ar*H*), 6.45 (dd, 1H, *J* = 5.40 Hz, Ar*H*), 3.65 (m, 4H, -N(C*H*$_2$)$_2$), 3.45 (t, 2H, *J* = 4.68 Hz, -SC*H*$_2$), 3.21 (m, 4H, -N(C*H*$_2$)$_2$), 2.96 (t, 2H, *J* = 4.56 Hz, -C*H*$_2$CO), 2.72 (s, 3H, C*H*$_3$), 2.38 (s, 3H, C*H*$_3$), 2.29 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 186.08, 176.20, 173.29, 153.29, 139.95, 138.15, 137.90, 132.40, 130.53, 128.82, 127.84, 126.73, 126.56, 122.77, 115.96, 51.49, 44.44, 35.13, 29.03, 19.77, 13.84, 13.12; ESI-MS: *m/z* 447.25; Anal. $C_{26}H_{28}N_2O_3S$: C, 69.54; H, 6.67; N, 83; Found: C, 69.62; H, 6.82; N, 6.73.

2-((3-(4-(4-bromo-2,5-dimethoxybenzyl)piperazin-1-yl)-3-oxopropyl)thio)-3 methyl naphthalene-1,4-dione (MB-18)

FT-IR (KBr, υ cm^{-1}): 3068.87 (aromatic C-H stretch), 2932.69 (aliphatic C-H stretch), 1725.66 (C=O stretch), 1689.28 (C=O stretch), 1247.91 (C-O stretch), 751.90; ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 8.19 (dd, 1H, J_m = 1.64 Hz, J_o = 5.52 Hz, Ar*H*), 8.10 (dd, 1H, J_m = 1.68 Hz, J_o = 5.48 Hz, Ar*H*), 7.79 (m, 2H, Ar*H*), 7.08 (s, 1H, Ar*H*), 6.81(s, 1H, Ar*H*), 3.80 (s, 6H, (OC*H*$_3$)$_2$), 3.71 (s, 2H, -N-C*H*$_2$), 3.53 (t, 2H, *J* = 4.16 Hz, N-

C*H*$_2$), 3.43 (t, 2H, *J* = 4.24 Hz, -N-C*H*$_2$), 3.30 (t, 2H, *J* = 4.16 Hz, -N-CH$_2$), 2.98 (t, 2H, *J* = 4.16 Hz, SC*H*$_2$), 2.82 (t, 2H, *J* = 6.0 Hz, -C*H*$_2$CO), 2.23 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 183.10, 177.92, 170.94, 153.10, 151.68, 139.39, 132.32, 133.33, 128.30, 127.29), 116.88, 113.56, 113.29, 110.19, 58.22, 56.78, 55.07, 54.61, 49.23 32.45, 32.27, 8.59; ESI-MS: *m/z* 572.42 (M$^+$), 574.42 (M$^+$ + 2); Anal. $C_{27}H_{29}BrN_2O_5S$: C, 56.80; H, 5.28; N, 4.64; Found: C, 56.88; H, 5.20; N, 4.72.

2-Methyl-3-((3-(4-(2-nitrophenyl)piperazin-1-yl)-3-oxopropyl)thio)naphthalene-1,4-dione (MB-19)

FT-IR (KBr υ cm^{-1}): 3067.95 (aromatic C-H stretch), 2931.94 (aliphatic C-H stretch), 1726.61 (C=O stretch), 1662.41 (C=O stretch), 1246.76 (C-O stretch), 812.09; ^{1}H NMR (CDCl$_3$, 400 MHz δ): 8.18 (dd, 1H, J_m = 1.65 Hz, J_o = 5.50 Hz, Ar*H*), 8.04 (dd, 1H, J_m = 1.68 Hz, J_o = 5.46 Hz, Ar*H*), 7.87 (m, 1H, Ar*H*), 7.62 (m, 2H, Ar*H*), 7.44 (m, 1H, Ar*H*), 6.97 (m, 1H, Ar*H*), 6.89 (m, 1H, Ar*H*), 3.65 (t, 2H, *J* = 4.44 Hz, -N-C*H*$_2$), 3.57 (t, 2H, *J* = 4.44 Hz, -N-C*H*$_2$), 3.34 (m, 6H, -N(CH$_2$)$_2$; -SC*H*$_2$), 3.08 (t, 2H, *J* = 4.56 Hz, -C*H*$_2$CO), 2.68 (s, 3H,C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 187.73, 177.54, 172.40, 145.31, 140.76, 139.30, 135.11, 137.62, 134.94, 133.84, 132.59, 128.15,127.48, 119.77, 118.15, 52.04, 44.84, 35.26, 29.36,12.40; ESI-MS: *m/z* 465.70; Anal. $C_{24}H_{23}N_3O_5S$: C, 61.37; H, 4.54; N, 9.92; Found: C, 61.65; H, 4.38; N, 9.45.

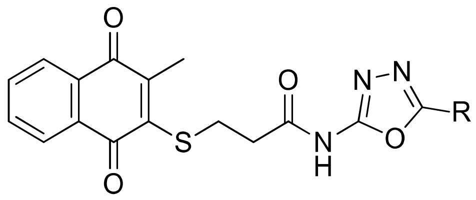
5.4. Characterization of Oxadiazoles substituted 1,4-Naphthoquinone (Series II)

The FT-IR spectrum of compounds (**MB-20** to **MB-33)** displayed a strong band at ~3400-3200 cm^{-1} corresponding to the N-H stretching vibrations. The aromatic nucleus in the structural skeleton of these compounds exhibited C-H stretching bands from 3100-3000 cm^{-1}. The C=N stretch for compounds **MB-20** to **MB-33** has been observed at ~1635 cm^{-1} in the IR spectra. Skeletal vibrations corresponding to C=C stretching within the aromatic ring appeared in the region of 1631-1600 cm^{-1}. The presence of the carboxylic acid functionality in all the synthesized compounds was confirmed by the disappearance of -OH band at 3358.31 cm^{-1} in the IR spectra. Appearance of a characteristic peak of the carbonyl group of entitled compounds was observed between 1706 to 1633 cm^{-1}. The presence of C-O-C function of the oxadiazole ring showed absorption in the range of 1150-1298 cm^{-1}. The disappearance of OH proton at δ 9.47 ppm in ^{1}H NMR spectra of the synthesized compounds was established by the substitution at the hydroxyl group. The aromatic protons resonated in the range of δ 8.21 to 5.64 ppm in the ^{1}H NMR spectra. Compound **MB-20** showed singlet of methyl group at δ 2.19 (CH_3) and compound **MB-21** depict one quartet at 2.57 (CH_2), and one triplet at δ 1.29 (CH_3). However, in compound **MB-22** two triplet and one multiplet and one triplet appeared at 2.67 (CH_2), 1.68 (CH_2) and 1.02 (CH_3). Compound **MB-24** to **MB-27** displayed the methoxy proton in the range of δ 3.78-3.80. A singlet of methyl group showed at δ 2.36 in compounds **MB-31** whereas in **MB-32** two singlet of methyl group observed at δ 2.43 and 2.32 respectively. In the compound **MB-33** a singlet of hydroxyl group and methyl group were observed at 9.45 and 2.32. In the ^{13}C NMR spectrum, carbonyl carbon of naphthalene ring was appeared at ≃δ 186.0 ppm and 175.0. Although aliphatic carbonyl, carbon observed at ≃ 170.0 ppm. The Signal of ^{13}C NMR spectrum further established the synthesis of the desired compounds with the appearance of aromatic carbons in the range from δ 162.00 to 101.00 ppm.

However the methylene carbon peak for the compound **MB-20** to **MB-33** showed between at δ 34.15-54.36 ppm. A prominent molecular ion peak along with isotopic peak of compound MB-28, MB-29 and MB-30 at m/z 453.17 (M^+), 455.17 ($M^+ + 2$), 453.12 (M^+), 455.12 ($M^+ + 2$) and 488.958 (M^+), 490.98 ($M^+ + 2$) were observed in ESI-MS spectrum and established the chemical structures.

The observed CHN analysis data was fully consistent with the calculated values that supported the proposed structures of the molecules. oxadiazole substituted 1,4-naphthoquinone derivatives and their physiochemical chemical properties are mentioned in **Table 5.2**.

Table1 5.2: Chemical structure of various 1,3,4- oxadiazole substituted 1,4-naphthoquinone derivatives (MB-20 to MB-33)



MB-20 to **MB-33**

Compounds	R	Molecular weight	Melting point (°C)	Appearance	Log P	% Yield	R_f
MB-20	CH_3-	357.38	220-222	White	0.77	58	0.48
MB-21	CH_2CH_3-	371.41	209-211	Brown	1.34	63	0.49
MB-22	$CH_2CH_2CH_3$-	385.44	183-185	White	1.76	52	0.50
MB-23	C_6H_5-	419.45	146-148	Yellow	2.15	46	0.55
MB-24	4-$OCH_3C_6H_5$-	449.48	215-217	Brown	2.03	49	0.54
MB-25	2-$OCH_3C_6H_5$-	449.48	230-232	Brown	2.03	62	0.54
MB-26	3-$OCH_3C_6H_5$-	449.48	205-207	White	2.03	58	0.54
MB-27	2,4-$(OCH_3)_2C_6H_5$-	479.51	221-223	White	1.9	49	0.52
MB-28	4-Cl- C_6H_5-	453.90	181-183	Brown	2.71	62	0.61
MB-29	2-ClC C_6H_5-	453.90	178-180	White	2.71	64	0.61
MB-30	2,3-$(Cl)_2C_6H_5$-	488.34	191-193	Yellow	3.27	57	0.72
MB-31	4-CH_3 C_6H_5-	433.48	157-159	White	2.64	56	0.58
MB-32	2,5-$(CH_3)_2C_6H_5$-	447.51	236-238	Creamy	3.13	63	0.63
MB-33	4-OH-3-$CH_3C_6H_5$-	449.48	213.215	Yellow	2.25	51	0.56

N-(5-methyl-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)propanamide (MB-20)

FT-IR (KBr, υ cm^{-1}): 3356.6 (N-H stretch), 3205.24 (aromatic C-H stretch), 2989.12 (aliphatic C-H stretch), 1673.80 (C=O stretch), 1634.35 (C=O stretch); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.48 (s, 1H, -*NH)*, 8.18 (m, 4H, Ar*H*), 2.98 (t, 2H, *J* = 5.64 Hz, -SCH_2), 2.85 (t, 2H, *J* = 5.48 Hz, - CH_2C=O), 2.77 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.73, 178.54, 170.21, 152.84, 151.50, 139.45, 137.50, 133.84, 132.40, 130.93, 127.90, 128.93, 34.04, 30.31, 15.11, 8.96; ESI-MS: m/z 357.08; Anal. $C_{17}H_{15}N_3O_4S$: C 54.32, H 3.73, N 28.79; Found: C54.83, H 3.12, N 28.83.

N-(5-ethyl-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2 yl)thio)propanamide (MB-21)

FT-IR (KBr, υ cm^{-1}): 3395.4 (N-H stretch), 3105.38 (aromatic C-H stretch), 2950.60 (aliphatic C-H stretch), 1686.90 (C=O stretch), 1644.61 (C=O stretch); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.54 (s, 1H, -N*H*), 8.19 (m, 2H, Ar*H*), 7.74 (m, 2H, Ar*H*) 3.18 (t, 2H, *J* = 5.48 Hz, -SCH_2), 2.85 (t, 2H, *J* = 5.52 Hz, -CH_2C=O), 2.77 (s, 3H, CH_3), 2.57 (q, 2H, *J* = 6.80 Hz, CH_2); 1.29 (t, 3H, *J* = 6.76 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.72, 178.83, 170.79, 155.28, 154.17, 139.45, 137.50, 133.83, 132.39, 130.80, 128.83, 127.89, 34.36, 30.20, 18.78, 15.97 and 7.14. ESI-MS: m/z 371.12; Anal. $C_{18}H_{17}N_3O_4S$: C, 58.21, H, 4.61, N, 11.31; Found: C, 58.93, H, 4.24, N, 11.39.

3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)-N-(5-propyl-1,3,4-oxadiazol-2-yl)propanamide (MB-22)

FT-IR(KBr, υ cm^{-1}): 3366.93 (N-H stretch), 3065.72 (aromatic C-H stretch), 2995.8 (aliphatic C-H stretch), 1703.31 (C=O stretch), 1658.69 (C=O stretch); ^{1}HNMR ($CDCl_3$, 400 MHz, δ): 9.54 (s, 1H, -N*H*), 8.19 (m, 2H, Ar*H*), 7.75 (m, 2H, Ar*H*), 3.20

(t, 2H, *J* = 5.52 Hz, -SCH$_2$), 2.86 (t, 2H, *J* = 5.52 Hz, -CH$_2$C=O), 2.78 (s, 3H, C*H*$_3$), 2.67 (t, 2H, *J* = 8.04 Hz, C*H*$_2$), 1.68 (m, 2H, C*H*$_3$) and 1.02 (t, 3H, *J* = 6.64 Hz, CH$_3$). ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.86, 178.77, 170.57, 156.77, 155.50, 139.44, 137.82, 133.58, 132.40, 130.38, 128.04, 127.90, 34.68, 30.97, 27.51, 18.78, 17.14, 15.32; ESI-MS: m/z: 385.09; Anal. $C_{19}H_{19}N_3O_4S$: C, 59.21, H 4.97, N, 25.83; Found: C, 59.64, H, 5.12, N, 25.43.

3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)propanamide (MB-23)

FT-IR(KBr, υ cm^{-1}): 3354.00 (N-H stretch), 3137.95 (aromatic C-H stretch), 2952.30 (aliphatic C-H stretch), 1671.92 (C=O stretch), 1644.14 (C=O stretch), 1540.16 (C=C ring stretch), 1446.46 (C-N stretch), 1268.15 (C-O stretch), 721.49 cm^{-1} (C-H bend); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 9.79 (s, 1H, -N*H*), 8.20 (dd, 1H, J_m = 1.52 Hz, J_o = 7.52 Hz, Ar*H*), 8.14 (dd, 1H, J_m = 1.52 Hz, J_o= 7.52 Hz, Ar*H*), 7.73 (m,1H, Ar*H),* 7.70 (m, 2H, Ar*H*) *7.63* (m, 1H*,* Ar*H*),7.45(m, 3H, Ar*H*), 3.16 (t, 2H, *J* = 5.60 Hz, -SC*H*$_2$), 2.86 (t, 2H, *J* = 5.64 Hz,- C*H*$_2$C=O) and 2.63 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.13, 178.04, 170.31, 155.80, 154.90, 139.23, 137.79, 133.51, 132.04, 130.31, 129.04, 128.13, 127.02, 127.52, 126.61, 34.19, 30.12, 15.86 (*C*H$_3$); ESI-MS: *m/z* 419.16; Anal. $C_{22}H_{17}N_3O_4S$: C, 63.02, H, 4.09, N, 10.02; Found: C, 63.43, H, 4.34, N, 10.25.

N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)thio)propanamide (MB-24)

FT-IR (KBr, υ cm^{-1}): 3329.72 (N-H stretch), 3178.02 (aromatic C-H stretch), 2941.58 (aliphatic C-H stretch), 1684.43 (C=O stretch), 1633.57 (C=O stretch); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 9.45 (s, 1H, -N*H*), 8.20 (m, 2H, Ar*H*),7.74 (m, 2H, Ar*H*), 7.61 (d, 2H *J* = 7.44 Hz, Ar*H*), 7.06 (d, 2H*, J* = 7.44 Hz, Ar*H*), 3.78 (s, 3H, -OC*H*$_3$), 3.07 (t, 2H, J = 5.88 Hz, -SCH_2), 2.90 (t, 2H, J = 5.84Hz, -CH_2C=O), 2.66 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.15, 178.21, 170.21, 162.15, 155.62, 154.93, 139.25, 137.86, 133.77, 132.77, 130.26, 128.41, 127.21, 127.89, 121.54, 113.87, 56.03, 34.73, 30.80, 15.18; ESI-MS: *m/z* 449.06; Anal. $C_{23}H_{19}N_3O_5S$: C, 61.46, H, 4.26, N, 9.35;Found: C, 61.29, H, 4.15, N, 9.96.

N-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)thio)propanamide (MB-25)

FT-IR (KBr, υ cm^{-1}): 3342.39 (N-H stretch), 3077.35 (aromatic C-H stretch), 2940.10 (aliphatic C-H stretch), 1685.95 (C=O stretch), 1640.92 (C=O stretch); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.67 (s, 1H, -NH), 8.08 (dd, 1H, J_m = 1.52, J_o = 7.48 Hz, ArH), 8.03 (dd, 1H, J_m = 1.52 Hz, J_o= 7.40 Hz, ArH), 7.77 (td, 1H, J_m= 1.52 Hz, J_o = 7.52 Hz, ArH), 7.56 (m, 2H, ArH), 7.35 (td, 1H, J_m = 1.44 Hz, J_o = 7.45 Hz, ArH), 7.12 (m, 2H, ArH), 3.80 (s, 3H, OCH_3), 3.29 (t, 2H, J = 5.20 Hz, -SCH_2), 2.89 (t, 2H, J = 5.16 Hz, -CH_2C=O), 2.56 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 186.31, 178.40, 170.30, 158.40, 155.87, 143.56, 139.47, 137.31, 133.05, 132.16, 131.09, 131.08, 130.58, 128.57, 127.89, 120.54, 118.87, 115.30, 56.03, 34.36, 30.37, 15.73; ESI-MS: *m/z* 449.13; Anal. $C_{23}H_{19}N_3O_5S$: C, 61.46, H, 4.26, N 9.35; Found: C, 61.12, H, 4.76, N, 9.27.

N-(5-(3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)thio)propanamide (MB-26)

FT-IR (KBr, υ cm^{-1}): 3370.97 (N-H stretch), 3188.49 (aromatic C-H stretch), 2979.23 (aliphatic C-H stretch), 1699.17 (C=O stretch), 1640.75 (C=O stretch); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.54 (s, 1H, -NH), 8.18 (m, 2H, ArH),7.76 (m, 2H, ArH), 7.47 (t, 1H, J = 7.48 Hz, ArH), 7.27 (m, 2H,ArH),7.12 (m, 1H, ArH), 6.92 (m, 1H, ArH), 3.79 (s, 3H, -OCH_3),3.01 (t, 2H, J = 5.88 Hz, -SCH_2), 2.86 (t, 2H, J = 5.80 Hz, -

C*H*$_2$C=O), 2.63 (s, 3H, C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.96, 178.28, 170.36, 161.17, 155.11, 153.42, 139.06, 137.94, 133.36, 132.84, 130.53, 128.54, 128.00, 127.82, 125.25, 120.51, 117.36, 114.11, 115.30, 56.03, 34.12, 30.82, 15.52; ESI-MS: *m/z* 449.02; Anal. $C_{23}H_{19}N_3O_5S$: C, 61.46, H, 4.26, N, 9.35; Found: C, 61.63, H, 4.81, N, 9.30.

N-(5-(2,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4 dihydro-naphthalen-2-yl)thio)propanamide (MB-27)

FT-IR (KBr, υ cm^{-1}): 3399.96 (N-H stretch), 3003.84 (aromatic C-H stretch), 2825.86 (aliphatic C-H stretch), 1706.45 (C=O stretch), 1649.17 (C=O stretch); ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 9.64 (s, 1H, -N*H*), 8.13 (m, 2H, Ar*H*), 7.79 (m, 2H, Ar*H*), 7.53 (m, 1H, Ar*H),* 6.74 (m, 2H,Ar*H*)*,* 3.80 (s, 6H, (OC*H*$_3$)$_2$)*,* 3.30 (t, 2H, *J* = 5.28 Hz, -SC*H*$_2$), 2.86 (t, 2H, *J* = 5.28 Hz, -C*H*$_2$CO), 2.42 (s, 3H, C*H*$_3$). ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.91, 178.73, 170.56, 161.96, 159.32, 155.88, 143.32, 139.60, 137.53, 133.49, 132.55, 130.47, 130.42, 128.43, 127.90, 116.17, 107.96, 101.25, 56.78, 56.03, 34.93, 30.31, 15.62; ESI-MS: *m/z* 479.88; Anal. $C_{24}H_{21}N_3O_6S$: C, 60.12, H, 4.41, N, 8.76; Found: C, 60.23, H, 4.33, N, 8.69.

N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)thio)propanamide (MB-28)

FT-IR (KBr, υ cm^{-1}): 3363.09 (N-H stretch), 3053.52 (aromatic C-H stretch), 2956.59 (aliphatic C-H stretch), 1681.59 (C=O stretch), 1643.92 (C=O stretch). ^{1}H NMR (CDCl$_3$, 400 MHz, δ): 9.70 (s, 1H, -N*H*), 8.19 (m, 2H, Ar*H*), 7.74 (m, 2H, Ar*H*), 7.48 (q, 4H, *J* = 7.52 Hz, Ar*H*), 3.17 (t, 2H, *J* = 8.16 Hz, SC*H*$_2$), 2.78 (t, 2H, *J* = 8.20 Hz, -C*H*$_2$CO), 2.70 (s, 3H, C*H*$_3$). ^{13}C NMR (CDCl$_3$, 100 MHz, δ): 185.55, 178.46, 170.55, 155.14, 154.55, 139.50, 137.84, 137.74, 133.64, 132.22, 130.49, 129.25, 128.85, 128.61, 128.59, 127.71, 34.02, 30.30, 15.22; ESI-MS: *m/z* 453.17 (M$^+$), 455.17 (M$^+$ +

2); Anal. $C_{22}H_{16}ClN_3O_4S$: C, 58.21, H, 3.55, N, 9.26; Found: C, 28.31, H, 3.48, N, 9.20.

N-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)thio)propanamide (MB-29)

FT-IR (KBr, υ cm^{-1}): 3340.63 (N-H stretch), 3171.42 (aromatic C-H stretch), 2931.64 (aliphatic C-H stretch), 1695.12 (C=O stretch), 1651.64 (C=O stretch); ^1H NMR ($CDCl_3$, 400 MHz, δ): 9.73 (s, 1H, -N*H*), 8.22 (m, 2H, Ar*H*), 7.73 (m, 2H, Ar*H*), 7.60 (m, 1H, Ar*H*), 7.43 (m, 1H, Ar*H*), 7.33 (m, 2H, Ar*H*), 3.16 (t, 2H, *J* = 8.20 Hz, SCH_2) 2.78 (t, 2H, *J* = 8.16 Hz, $-CH_2CO$), 2.74 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.93, 178.27, 170.18, 155.60, 142.35, 139.96, 137.12, 134.28, 133.17, 132.11, 131.94, 131.06, 130.36, 128.84, 127.56, 127.60, 127.00, 126.54, 34.92, 30.25, 15.61; ESI-MS: *m/z* 453.12 (M^+), 455.12 ($M^+ + 2$); Anal. $C_{22}H_{16}ClN_3O_4S$: C, 58.21, H, 3.55, N, 9.26; Found: C, 58.28, H, 3.49, N, 9.30.

N-(5-(2,3-dichlorophenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)thio)propanamide (MB-30)

FT-IR (KBr, υ cm^{-1}): 3408.02 (N-H stretch), 3176.65 (aromatic C-H stretch), 2940.16 (aliphatic C-H stretch), 1686.75 (C=O stretch), 1634.00 (C=O stretch); ^1H NMR ($CDCl_3$, 400 MHz, δ): 9.87 (s, 1H, -N*H*), 8.25 (m, 2H, Ar*H*), 7.78 (m, 2H, Ar*H*), 7.37 (m, 2H, Ar*H*), 7.24 (m,1H, Ar*H*), 3.11 (t, 2H, *J* = 8.00 Hz, SCH_2), 2.82 (t, 2H, *J* = 7.92 Hz, $-COCH_2$), 2.65 (s, 3H, $-CH_3$. ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.30, 178.67, 170.65, 155.26, 141.87, 139.21, 137.39, 135.95, 134.91, 133.37, 132.76, 131.27, 130.36, 128.67, 128.64, 128.11, 127.67, 124.03, 34.32, 30.53, 15.08; ESI-MS: *m/z* 488.958 (M^+), 490.98 ($M^+ + 2$); Anal. $C_{22}H_{15}Cl_2N_3O_4S$. C, 51.56, H, 2.42, N, 18.72; Found: C, 51.86, H, 2.37, N, 18.23.

3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl)propanamide (MB-31)

FT-IR (KBr, υ cm^{-1}): 3372.78 (N-H stretch), 3032.03 (aromatic C-H stretch), 2971.35 (aliphatic C-H stretch), 1698.93 (C=O stretch), 1654.29 (C=O stretch); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.79 (s, 1H, -N*H*), 8.16 (m, 2H, Ar*H*), 7.71 (td, 1H, J_m = 1.40 Hz, J_o= 7.48 Hz, Ar*H*), 7.55 (m, 3H, Ar*H*), 7.32 (d, 2H, *J* = 5.64 Hz, Ar*H*), 3.17 (t, 2H, *J* = 5.64 Hz, SCH_2), 2.87 (t, 2H, *J* = 5.64 Hz, -CH_2CO), 2.63 (s, 3H, -CH_3), 2.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.61, 178.80, 170.00, 155.38, 154.36, 140.51, 139.64, 137.07, 133.67, 132.33, 130.28, 129.08, 128.16, 127.33, 126.83, 125.39, 40.91, 34.80, 30.57, 15.21; ESI-MS: *m/z* 433.04; Anal. $C_{23}H_{19}N_3O_4S$: C, 63.73, H, 4.42, N, 9.69; Found: C, 63.42, H, 4.47, N, 9.74.

N-(5-(2,5-dimethylphenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4 dihydro-naphthalen-2-yl)thio)propanamide (MB-32)

FT-IR (KBr, υ cm^{-1}): 3325.45 (N-H stretch), 3048.77 (aromatic C-H stretch), 2912.00 (aliphatic C-H stretch), 1694.48 (C=O stretch), 1648.72 (C=O stretch); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.59 (s, 1H, -N*H*), 8.18 (m, 2H, Ar*H*), 7.73 (m, 2H, Ar*H*), 7.47 (d, 1H, *J* = 1.40 Hz, Ar*H),* 7.18 (m, 2H,Ar*H*), 3.07 (t, 2H, *J* = 5.92 Hz, -S*C*H_2), 2.90 (t, 2H, *J* = 5.88 Hz, -CH_2C=O), 2.66 (s, 3H, -SCH_3), 2.43 (s, 3H, CH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.80, 178.54, 170.86, 155.79, 148.28, 139.45, 137.98, 137.38, 136.49, 133.82, 132.41, 130.58, 130.57, 128.75, 128.55, 128.53, 127.84, 34.09, 30.65, 121.19, 121.23, 15.98; ESI-MS: *m/z* 447.14; Anal. $C_{24}H_{21}N_3O_4S$: C, 64.41, H, 4.73, N, 9.39; Found: C, 64.53, H, 4.64, N, 9.33.

N-(5-(4-hydroxy-3-methylphenyl)-1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)propanamide (MB-33)

FT-IR (KBr, υ cm^{-1}): 3460.26 (O-H stretch), 3321.06 (N-H stretch), 3109.26 (aromatic

C-H stretch), 2984.64 (aliphatic C-H stretch), 1701.34 (C=O stretch), 1667.00 (C=O stretch), 1563.58(C=C ring stretch), 1482.75 (C-N stretch), 1282.68 (C-O stretch), 740.03 (C-H bend). ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 9.45 (s,1H, -N*H*), 8.18 (m, 3H, Ar*H*), 7.74 (m, 2H, Ar*H*), 7.28 (m, 2H, Ar*H*), 6.85 (s,1H, O*H*), 3.14 (m, 2H, SC*H*$_2$), 2.83 (t, 2H, *J* = 5.60 Hz, -C*H*$_2$C=O), 2.71 (s, 3H, -C*H*$_3$), 2.32 (s, 3H, -C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 185.42, 178.74, 170.01, 157.42, 155.23, 154.21, 139.10, 137.27, 133.38, 132.19, 130.20, 128.76, 127.98, 127.08, 126.67, 125.56, 117.51, 112.97, 34.25, 30.58, 16.01, 15.29. ESI-MS: *m/z* 449.93; Anal. $C_{23}H_{19}N_3O_5S$: C, 61.46, H, 4.26, N, 9.35; Found: C, 61.86, H, 4.23, N, 9.38.

5.5. Molecular Docking

Docking, the accurate prediction of binding mode of small molecule ligands to protein targets is central to numerous biological processes and is of fundamental importance in modern structure-based drug design. This has been exploited by medicinal chemists not only to predict both ligand orientation and binding affinity while designing molecules selectively to augment or retard biochemical pathways or to exhibit a clinical effect, but to meet the primary challenge in lead discovery and optimization process. Docking simulations (in silico) studies were performed on the compounds **MB-1** to **MB-33** using extra precision GRIP docking (vLifeMDS 4.6 software) in order to postulate a hypothetical binding model for their interaction with active site of epidermal growth factor receptor (EGFR) receptors.

Table 5.3 described molecular docking score of all the synthesized compounds. The analysis of the docking score of the pharmacologically active molecules suggested an appreciable placement of the molecule into the active site. The dock score analysis indicated that the synthesized compounds aligned themselves in a proper orientation in the cavity space suggesting a plausible explanation for the efficient fit of the molecules into the active site. Thus docking studies were done on the epidermal growth factor receptor (2GS6). These studies suggested that superior protein-ligand binding of synthesized compounds with key amino acid of the active site. Methionine residues (MET 793), cytosine (CYS797) and tyrosine (TRY253) are the key residues of the protein-ligand binding. These amino acid residues play a key role in the formation of a bridge within EGFR. Moreover, docking examinations of **MB-1** to **MB-33** on EGFR demonstrated a considerable binding interaction of the synthesized compound with the key amino acid residue. All the compounds have been found to possess a good binding affinity with the EGFR and afforded high dock score from **-**54.10 to -75.25. Grip docked pose of the fitted ligands were visualized extending deep into the active site pocket and showing several interactions such as Vander Waal's, hydrophobic contacts, hydrogen bonds and π-π stacking interactions with the key residues of the active site catalytic site as well as peripheral site. The standard drug imatinib form four hydrophobic interactions with the SER696, LEU834 and LYS851along with the distance 3.874 Å, 3.856 Å, 3.735 Å and 3.993 Å **Figure 5.1**.

In the series of piperazine substituted 1,4-naphthoquinone, compound **MB-9** attributed to its hydrogen bond interactions with amino acid residue ALA 698, to the piperazine nitrogen along with distance 2.338Å. Furthermore it forms hydrophobic interactions with ALA698, GLY697, PHE699 and VAL702 with distanced 3.953 Å, 4.165 Å, 4.34 Å and 2.522 Å. As well it formed π-π stacking interactions with TYRS with 5.111 Å distanced. However the compound **MB-18** formed hydrophobic interactions with amino acid residue of VAL702A, LEU694A,GLY695A, and SER696A with distance 3.07 Å, 3.942 Å, 3.905 Å, 3.31 Å respectively. Amongst all the docked ligands, compounds **MB-9** and **MB-18** has shown a highest binding affinity dock score with EGFR. The interactions of the active compounds of the piperazine substituted derivatives *viz.* **MB-9** and **MB-18** is clearly indicated in **Figure 5.2 a-c.**

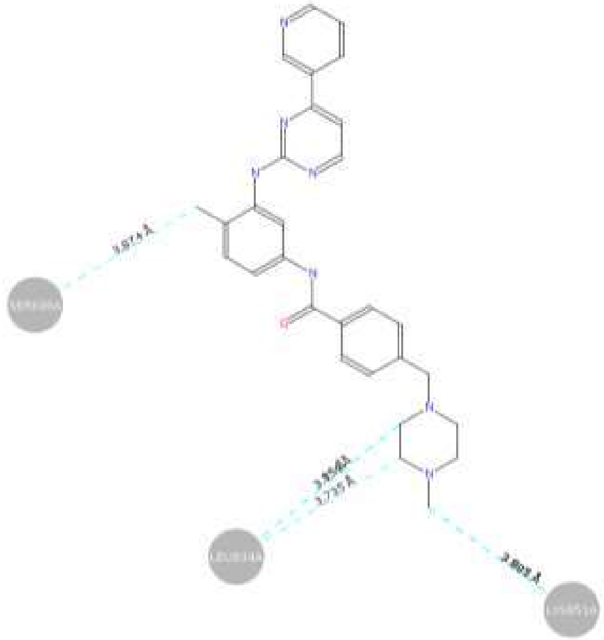
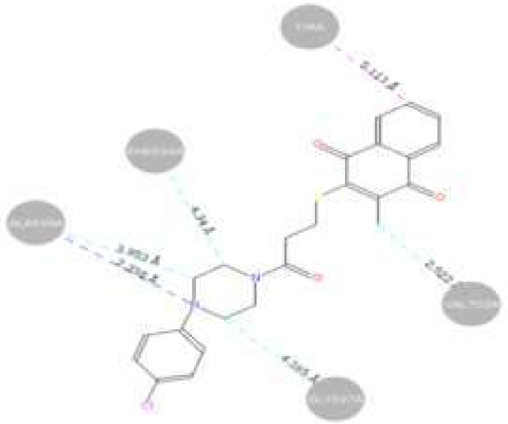
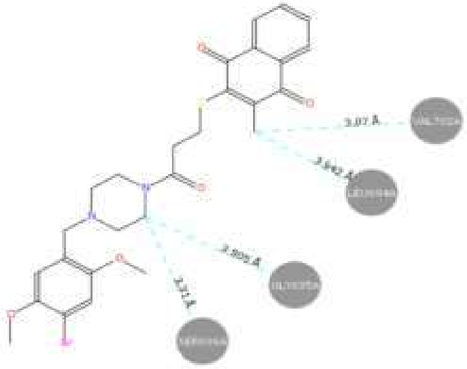


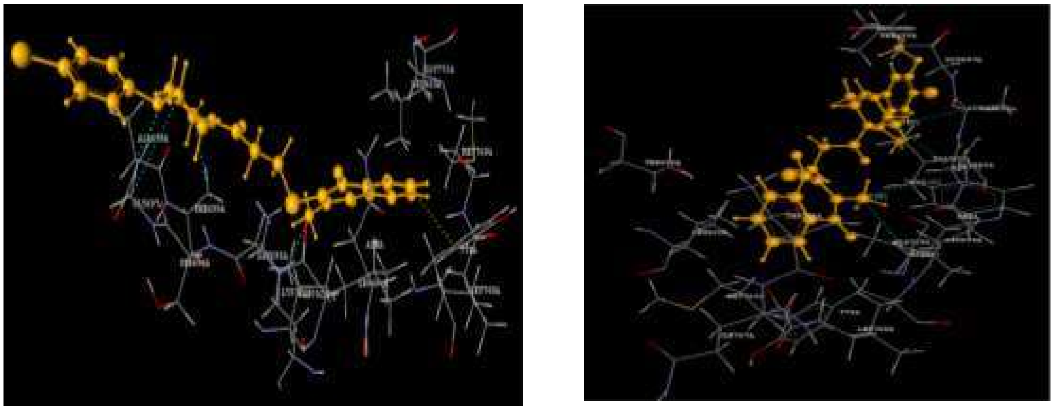
Figure 5.1: 2D hydrogen bonding interaction of standard drug imatinib



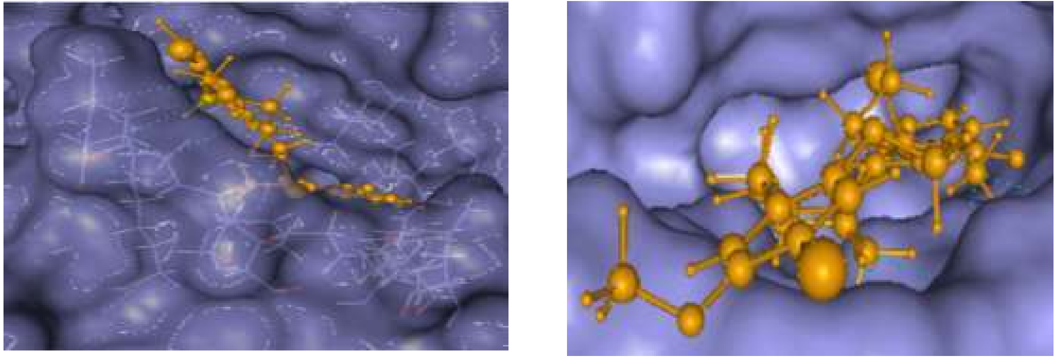
MB-9 (a)



MB-18 (a)



MB-9 (b) **MB-18 (b)**



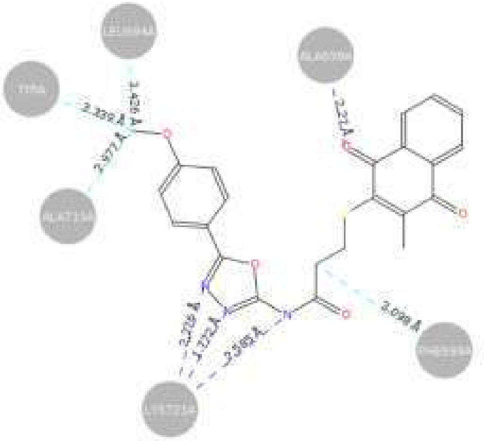
MB-9 (c) **MB-18 (c)**

Figure 5.2: (a) 2D hydrogen bond interaction, (b) 3D Hydrogen Bonding Interaction showing the binding orientation, (c) 3D Surface and Hydrogen Bonding Interaction of the compounds MB-9 and MB-18 into the active site of 2GS6
----------: Hydrophobic Interactions, ----------: Hydrogen Bonding

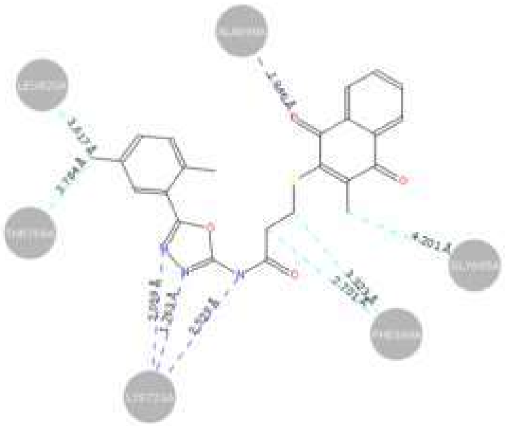
Similarly, in 1,3,4 oxadiazole substituted 1,4-naphthoquinone derivatives two compound **MB-24** and **MB-32** showed highest mocking score. Compound **MB-24** attributed strong hydrogen bonds between amino acid residue ALA698 to the oxygen of ring naphthoquinone ring with distance 2.22 Å and nitrogen of Oxadiazoles ring with LYS 721 along with distance 2.276, 1.772 and 2.585 Å respectively. Compound **MB-32** formed hydrogen bond with ALA698 amino residue of oxygen of naphthoquinone ring with distance 1.946 and and nitrogen of oxadiazole ring with LYS 721 amino residue through distance 2.059 Å, 1.763 Å and 2.528 Å. The results of the binding studies of the compound **MB-24** and **MB-32** have been represented in **Figure 5.3 a-c.**

The compound **MB-24** formed hydrophobic interaction with amino acid residues LEU694, TYRA, ALA719 and PHE 699 with the distance 3.426 Å, 3.339 Å, 2.977 Å and 3.098 Å respectively.

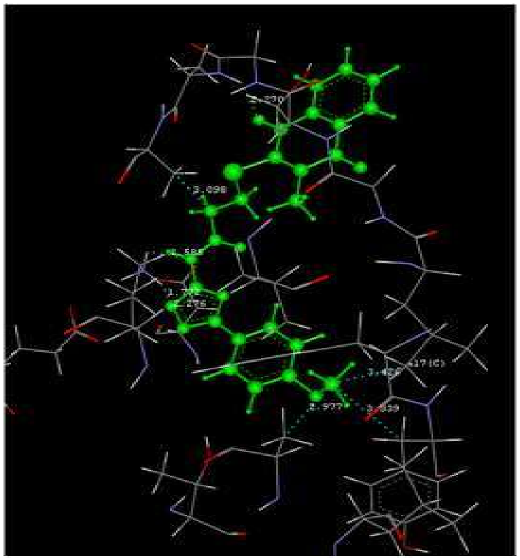
However, the compound **MB-32** have been formed hydrophobic interactions with LEU820, THR766, PHE699 and GLY 695 along with distance 3.617 Å, 3.784 Å, 2.701 Å, 3.323 Å, and 4.201 Å of same amino acid. Therefore, compounds 1,4-naphthoquinone derivatives formed considerable interactions with essential amino acid residues and revealed association of the docking studies with anticancer activity of the compounds.



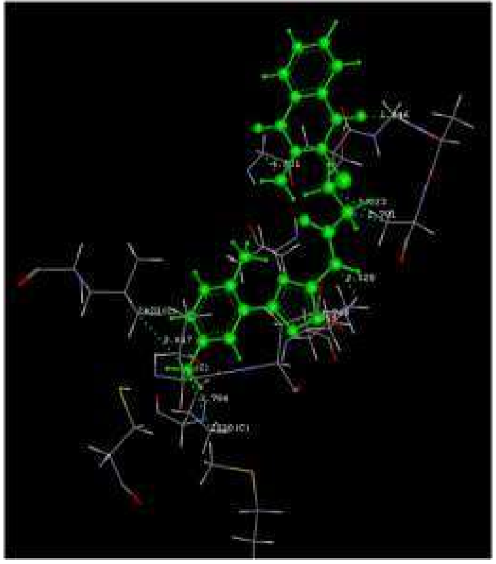
MB-24 (a)



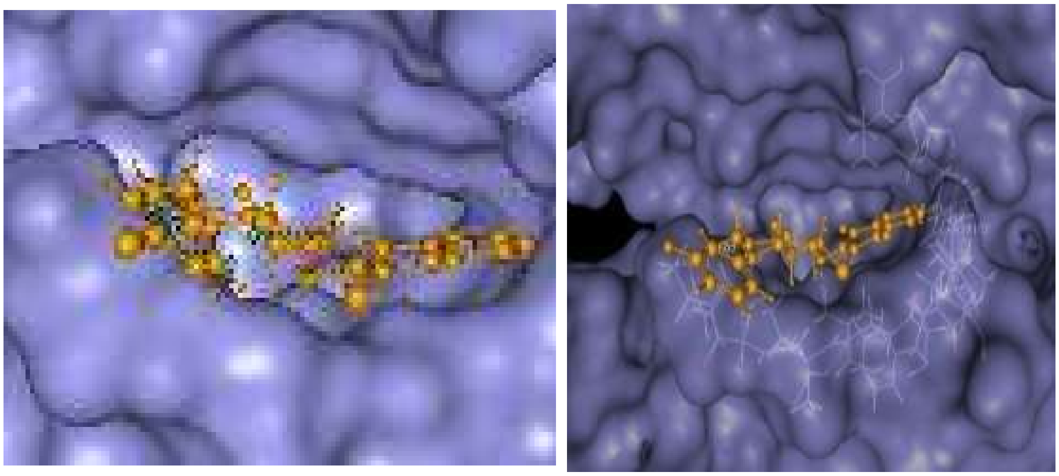
MB-32 (a)



MB-24 (b)



MB-32 (b)



MB-24 (c) **MB-32 (c)**

Figure 5.3: (a) 2D hydrogen bond interaction, (b) 3D Hydrogen Bonding Interaction showing the binding orientation, (c) 3D Surface and Hydrogen Bonding Interaction of the compounds MB-24 and MB-32 into the active site of 2GS6.

----------: Hydrophobic Interactions, ----------: Hydrogen Bonding

Table 5.3: Molecular docking score of compounds (**MB-1** to **MB-33**)

Compound	D-Score	Compound	D-Score
MB-1	-57.26	**MB-18**	-75.25
MB-2	-63.22	**MB-19**	-66.57
MB-3	-54.10	**MB-20**	-50.71
MB-4	-61.74	**MB-21**	-58.37
MB-5	-67.07	**MB-22**	-58.36
MB-6	-61.39	**MB-23**	-59.75
MB-7	-64.67	**MB-24**	-67.04
MB-8	-61.69	**MB-25**	-59.89
MB-9	-70.47	**MB-26**	-61.87
MB-10	- 65.72	**MB-27**	-56.20
MB-11	-57.42	**MB-28**	-57.07
MB-12	-58.45	**MB-29**	- 56.77
MB-13	-63.19	**MB-30**	-60.16
MB-14	-57.50	**MB-31**	-54.67
MB-15	-56.55	**MB-32**	-65.38
MB-16	-61.09	**MB-33**	-63.34
MB-17	-60.25	**Imatinib**	-62.92

5.6. *In-Silico* Pharmacokinetic Studies

In silico pharmacokinetic parameters prediction is one of the vital area in the development of new drug molecules. ADME (Absorption, Distribution, Metabolism and Excretion) properties of the synthesized compounds (**MB-9 and MB-24)** were predicted using QikProp program, version 3.5 (Schrodinger, LLC, New York, USA). This method is very robust and can estimate the physicochemical properties and the Drug-Likeness of the compounds. All descriptors have been calculated and illustrated in **Table 5.4.** The results concluded that the target compounds had molecular weight less than 500, logP equal to or less than 6.5 and 100 % human oral absorption showing the Drug-Likeness of the synthesized compounds. Solvent accessible surface area and vander Waals surface area of polar nitrogen and oxygen atoms are other key properties to have better drug bioavailability. The results revealed that molecules possessing a small vander Waals surface area of polar nitrogen and oxygen atoms may support governing forces in transiting biological membranes, resulting in good absorption favoring blood brain barrier (BBB) penetration. On this basis, the compounds having physicochemical properties under the acceptable criteria have been found to have drug like characteristics.

Table 5.4: QikProp analysis of compound MB-9, MB-24, MB-18 and MB-32

Parameters	**Imatinib**	**MB-9**	**MB-24**	**MB-18**	**MB-32**
Mol Wt[a]	493.61	454.97	449.48	545.447	447.508
Donor HB[b]	2	0	1	0	1
Acceptor HB[c]	10.5	8.5	10.25	10	9.5
SASA[d]	905.42	735.75	770.612	798.979	788.419
QPlogBB[e]	-0.451	-0.614	-1.917	-0.914	-1.637
QPlogPo/w[f]	3.54	3.18	2.626	3.233	3.219
QPPMDCK[g]	25.26	1325.9	133.245	1071.07	142.422
QPlogKhsa[h]	0.535	-0.261	-0.091	-0.356	0.233

[a] MolWt - molecular weight of the molecule (130-725).

[b] Donor HB -number of hydrogen bonds (0.0 - 6.0).

[c] AcceptorHB- number of hydrogen bonds (2.0 - 20.0).

[d] SASA- Total solvent accessible surface area in square angstroms using a probe with 1.4 Å radius (300-1000).

[e] QPlogBB - predicted brain/blood partition coefficient (-3.0 - 1.2).

[f] QPlogPo/w - this gives the predicted octanol/water partition coefficient (-2.0 - 6.5).

[g] QPPMDCK- predicted MDCK cell permeability in nm/s using the affix scale (< 25 is considered poor and > 500 is considered excellent).

[h] QPlogKhsa- prediction of binding to human serum albumin (-1.5 – 1.5).

5.7. Pharmacological Evaluation

5.7.1. *In-vitro* anticancer activity

All the newly synthesized compounds **MB-1** to **MB-33** were evaluated *in-vitro* to assess their anticancer potency using various human cancer cells such as breast carcinoma (MCF-7), liver carcinoma (HepG2) and cervical carcinoma (HeLa) cell lines using imatinib as standard drug. *In-vitro* antiproliferative activity, suggested that the cell c was found in the dose-dependent manner and most of the compound possessed the significant anticancer activity against these three cancer cell lines. Cell viability decreased and cell inhibition increases with increase in the concentration of compounds. Moreover the synthesized compounds exposed the cytotoxicity poor to strong comparatively to the standard drugs. The anticancer activity of the compound **MB-1** to **MB-19** and standard drug imatinib is expressed as IC_{50} depicted in the **Table 5.5**. In the synthesized derivatives of piperazine substituted 1,4-naphthquinones compound **MB-9** and **MB-18** revealed stronger activity than Imatinib (standard). The electron withdrawing chlorine group and the methoxy subtituents on the benzene ring attached to the piperazine ring increased the cytotoxicity on HeLa cells. Therefore, compound **MB-9** and **MB-18** from the series of piperazine substituted 1,4-naphthoquinone exhibited most promising anticancer activity against MCF-7, HepG2 and HeLa cell lines as compared to standard drug imatinib. The graphical representation of the data of all the tested compounds has been illustrated in **Figure 5.4**. Similarly in other series oxadiazole 1, 4-naphthoquinone compounds, **MB-24**, and **MB-32** exhibits significant anticancer activity against these three cancer cell lines. The anticancer activity of the compound **MB-20** to **MB-33** and standard drug imatinib is expressed as IC_{50} depicted in the **Table 5.6; Figure 5.5**. Thus in this series of compound **MB-24** and **MB-32** exhibited strong antiproliferative activity.

Table 5.5.: *In-vitro* cytotoxicity of piperazine substituted 1,4-naphthoquinone derivative (MB-1 to MB-19).

Compounds	HeLa cell IC_{50} (µM) ± SEM	MCF-7 cell IC_{50} (µM) ± SEM	HepG2 cell IC_{50} (µM) ± SEM
MB-1	76.73 ± 0.32*	71.53 ± 0.96*	89.63 ± 0.87*
MB-2	67.32 ± 0.19*	41.74 ± 0.98*	27.53 ± 0.32*
MB-3	54.42 ± 0.64$^{\#}$	67.40 ± 1.03*	28.73 ± 0.88*
MB-4	24.98 ± 0.62$^{\#}$	27.34 ± 0.23$^{\#}$	31.13 ± 0.39*
MB-5	29.56 ± 0.67$^{\#}$	51.21 ± 0.41$^{\#}$	34.71 ± 0.30*
MB-6	76.35 ± 0.98$^{\#}$	39.37 ± 0.50$^{\#}$	28.32 ± 0.85$^{\#}$
MB-7	91.44 ± 0.45$^{\#}$	94.39 ± 0.61*	83.80 ± 0.63$^{\#}$
MB-8	89.43 ± 0.67*	85.43 ± 0.32$^{\#}$	92.50 ± 1.21$^{\#}$
MB-9	13.45 ± 0.48$^{\#}$	15.63 ± 0.47$^{\#}$	23.87 ± 0.59$^{\#}$
MB-10	30.96 ± 0.41$^{\#}$	25.35 ± 0.22$^{\#}$	27.41 ± 0.55*
MB-11	37.87 ± 0.80*	28.91 ± 0.94$^{\#}$	28.20 ± 1.02*
MB-12	78.33 ± 0.75*	23.94 ± 0.38$^{\#}$	24.12 ± 0.74*
MB-13	23.52 ± 0.61$^{\#}$	27.30 ± 0.28$^{\#}$	39.12 ± 0.37*
MB-14	38.19 ± 0.52$^{\#}$	49.53 ± 1.31*	52.95 ± 0.61$^{\#}$
MB-15	75.86 ± 0.41*	28.24 ± 0.82*	20.32 ± 0.73$^{\#}$
MB-16	38.13 ± 0.29*	89.43 ± 1.03$^{\#}$	91.53 ± 0.75$^{\#}$
MB-17	48.53 ± 0.67$^{\#}$	26.72 ± 0.81$^{\#}$	24.35 ± 0.74$^{\#}$
MB-18	19.29 ± 0.22*	16.96 ± 0.21*	19.53 ± 0.39*
MB-19	34.33 ± 0.81*	24.19 ± 0.82$^{\#}$	29.74 ± 0.94*
Imatinib	21.38 ± 0.76	23.16 ± 0.30	24.48 ± 0.71

The results are expressed as mean ± SEM (n=3) and analyzed by one-way ANOVA Tukey's test (*) $p < 0.05$, (#) $p < 0.01$, statistical significant as compared to imatinib.

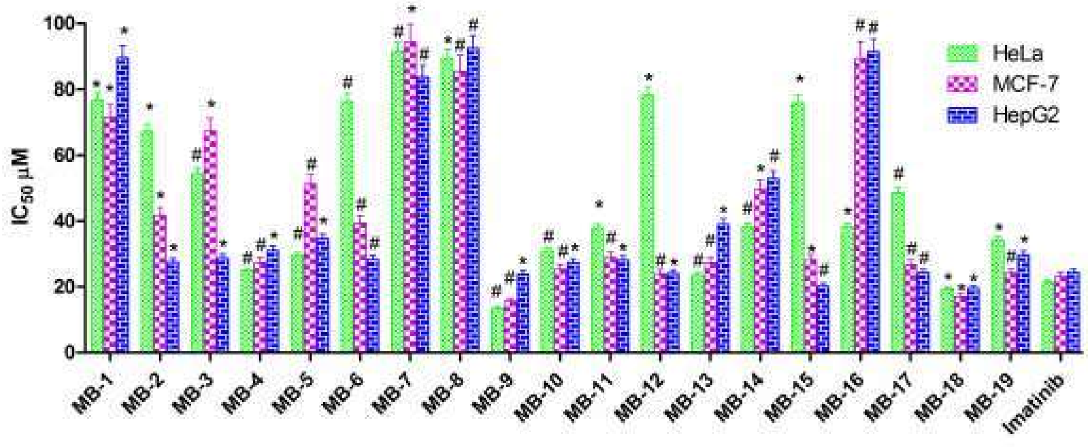


Figure 5.4: *In-vitro* cytotoxicity activity (IC_{50}) of compounds against HeLa, MCF-7 and HepG2 cancer cell line. The results are expressed as mean ± SEM (n=3). Significant values were determined by one-way ANOVA, followed by Tukeys test (*) p < 0.05, (#) p < 0.01, statistical significant as compared to imatinib.

Table 5.6: *In-vitro* cytotoxicity of oxadiazole substituted 2-methyl 1, 4-naphthoquinone compounds (**MB-20 to MB-33**).

Compounds	**HeLa cell IC_{50} (μM) ± SEM**	**MCF-7 cell IC_{50} (μM) ± SEM**	**HepG2 cell IC_5 (μM) ± SEM**
MB-20	55.65 ± 1.32*	60.83 ± 0.88*	58.64 ± 0.74*
MB-21	39.64 ± 0.19*	46.34 ± 0.63$^{\#}$	38.34 ± 1.84*
MB-22	53.73 ± 0.84*	63.92 ± 1.15$^{\#}$	46.72 ± 0.73$^{\#}$
MB-23	23.82 ± 0.66*	28.24 ± 0.85*	34.12 ± 0.92*
MB-24	10.76 ± 0.11$^{\#}$	9.30 ± 0.14$^{\#}$	11.93 ± 0.38$^{\#}$
MB-25	54.34 ± 0.98$^{\#}$	40.30 ± 0.82$^{\#}$	31.62 ± 0.45*
MB-26	87.44 ± 0.45$^{\#}$	83.80 ± 0.39$^{\#}$	61.93 ± 0.68$^{\#}$
MB-27	64.47 ± 1.85$^{\#}$	85.16 ± 0.54*	64.63 ± 0.87*
MB-28	20.44 ± 0.85$^{\#}$	26.82 ± 0.83*	23.80 ± 0.67$^{\#}$
MB-29	28.85 ± 0.71$^{\#}$	28.25 ± 0.53$^{\#}$	29.48 ± 0.25$^{\#}$
MB-30	32.93 ± 0.86$^{\#}$	30.51 ± 0.89$^{\#}$	31.73 ± 0.92$^{\#}$
MB-31	56.79 ± 0.71$^{\#}$	21.73 ± 0.18$^{\#}$	21.82 ± 0.54$^{\#}$
MB-32	13.23 ± 0.27*	15.30 ± 0.42*	17.39 ± 0.17$^{\#}$
MB-33	54.32 ± 0.68*	38.53 ± 0.85*	50.90 ± 0.26*
Imatinib	21.38 ± 0.76	23.16 ± 0.30	24.48 ± 0.71

The results are expressed as mean ± SEM (n=3) and analyzed by one-way ANOVA Tukey's test (*) $p < 0.05$, (#) $p < 0.01$, statistical significant as compared to imatinib.

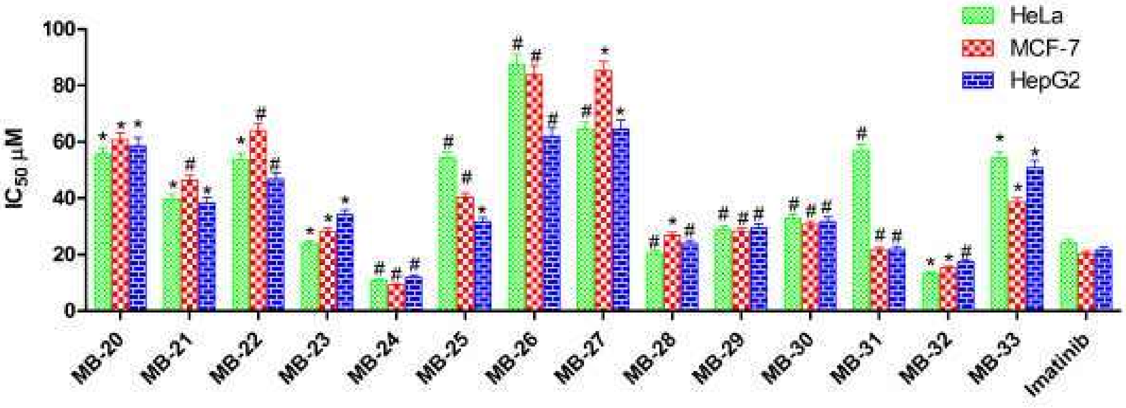


Figure 5.5: *In-vitro* cytotoxicity activity (IC_{50}) of compounds against HeLa, MCF-7 and HepG2 cancer cell line. The results are expressed as mean ± SEM (n=3). Significant values were determined by one-way ANOVA, followed by Tukeys test (*) $p < 0.05$, (#) $p < 0.01$, statistical significant as compared to imatinib.

5.8. *In-vitro* EGFR Kinase Inhibition

The inhibitory activity of the synthesized compound (**MB-1** to **MB-33**) against EGFR kinase has been evaluated *in-vitro* according to the method (Hu *et al.,* 2017). The percentage inhibition of the synthesized compounds with standard imatinib was calculated at five different concentrations (1-15µM). The concentration *vs.* percentage inhibition graph was plotted and IC_{50} values were determined graphically by extrapolation. The estimation of the IC_{50} values supported a noticeable analysis of the inhibitory activity of the compounds against EGFR tyrosine kinase. Amongst the series of piperazine substituted 1, 4-naphthoquinone derivatives, compounds **MB-9, MB-13** and **MB-18** exhibited promising inhibitory potential with the IC_{50} values of 1.80 ± 0.06 µM, 2.43 ± 0.11 µM, and 2.03 ± 0.0 7respectively (**Table 5.7**). Thus this series of compounds **MB-9** and **MB-18** contain most potent inhibition activity against the enzyme EGFR kinase. The graphical representation of the data (IC_{50}) of all the tested compounds has been illustrated in **Figure 5.6.**

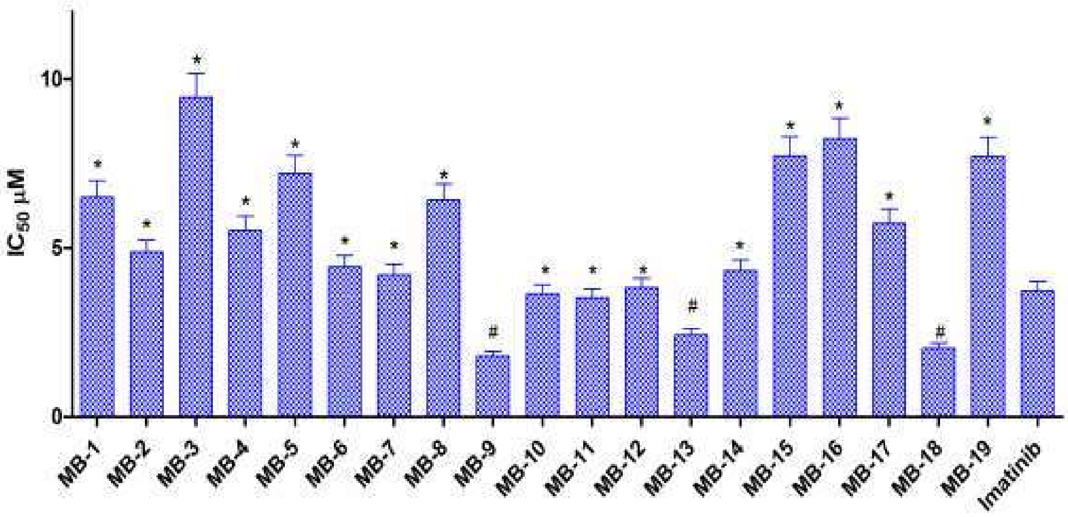


Figure 5.6: *In-vitro* inhibitory activity (IC_{50}) of compounds against EGFR kinase. The statistically significant was determined by one-way ANOVA, followed by Tukeys test (*) $p < 0.05$ and (#) $p < 0.01$ significant as compared to imatinib.

Table 5.7: *In-vitro* inhibitory activities (IC_{50}) of synthesized compound (MB-20 to MB-33), against EGFR tyrosine kinase.

Compounds	Tyrosine kinase inhibition IC_{50} (μM) ± SEM	Compounds	Tyrosine kinase inhibition IC_{50} (μM) ± SEM
MB-1	6.51 ± 0.48*	**MB-18**	2.03 ± 0.07$^{\#}$
MB-2	4.88 ± 0.45*	**MB-19**	7.71 ± 0.38*
MB-3	9.46 ± 0.26*	**MB-20**	5.32 ± 0.15*
MB-4	5.53 ± 0.22*	**MB-21**	4.16 ± 0.13*
MB-5	7.21 ± 0.21*	**MB-22**	7.23 ± 0.16*
MB-6	4.45 ± 0.23*	**MB-23**	5.43 ± 0.10*
MB-7	4.21 ± 0.20*	**MB-24**	1.53 ± 0.05$^{\#}$
MB-8	6.42 ± 0.15*	**MB-25**	4.74 ± 0.12*
MB-9	1.80 ± 0.06$^{\#}$	**MB-26**	4.66 ± 0.11*
MB-10	3.64 ± 0.18*	**MB-27**	5.80 ± 0.16*
MB-11	3.73 ± 0.10*	**MB-28**	5.36 ± 0.15*
MB-12	3.82 ± 0.14*	**MB-29**	4.62 ± 0.19*
MB-13	2.43 ± 0.11$^{\#}$	**MB-30**	3.65 ± 0.12$^{\#}$
MB-14	4.33 ± 0.14*	**MB-31**	3.98 ± 0.12*
MB-15	7.72 ± 0.13*	**MB-32**	2.54 ± 0.10#
MB-16	8.24 ± 0.17*	**MB-33**	4.64 ± 0.13*
MB-17	5.73 ± 0.11*	**Imatinib**	3.54 ± 0.11

All the values are expressed as mean ± SEM (n=3). The statistically significant values were determined by one-way ANOVA, followed by Tukeys test (*) $p < 0.05$ and (#) $p < 0.01$ significant as compared to imatinib

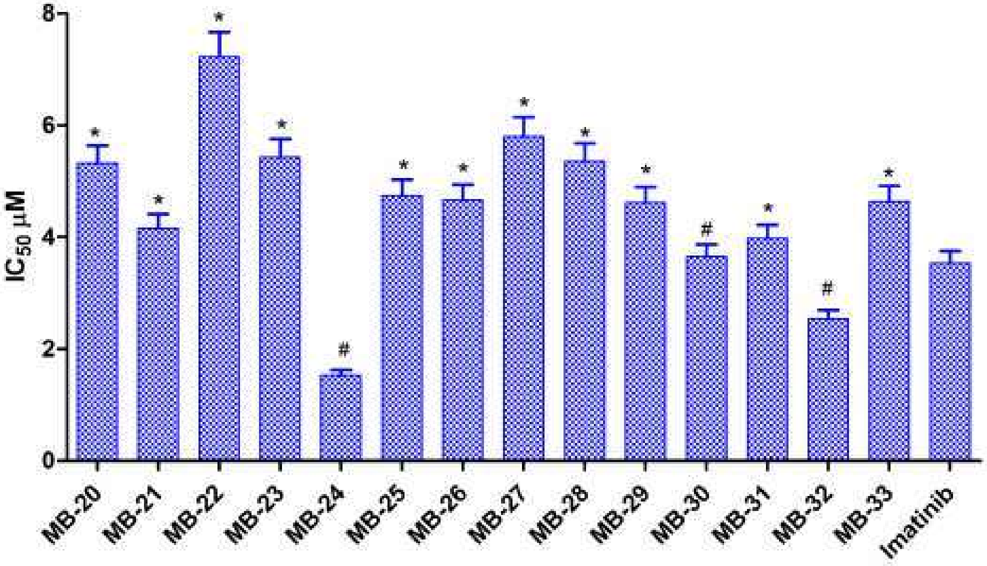


Figure 5.7: *In-vitro* inhibitory activity (IC_{50}) of compounds against against EGFR kinase. The statistically significant was determined by one-way ANOVA, followed by Tukeys test (*) $p < 0.05$ and (#) $p < 0.01$ significant as compared to imatinib.

In another series of oxadiazole substituted 1,4-naphthoquinone derivative, compounds **MB-24** and **MB-32** demonstrated significant tyrosine kinase inhibition with minimum IC_{50} values of 1.53 ± 0.05 µM and 2.54 ± 0.10 µM, respectively (**Table 5.7**). The most promising compounds (**MB-24** and **MB-32)** of this series were found to be approximately 2.3 times more inhibition than standard drug Imatinib (IC_{50} = 3.54 ± 0.11 µM), **(Figure 5.7)**.

5.9. *In-vivo* Anticancer Activity Evaluation

The compounds MB-9 and MB-18 from series of piperazine substituted 1,4-naphthoquinone and compound MB-24 and MB-32 from series of substituted 1,4-naphthoquinone showed significant cytotoxicity against MCF-7 (human breast cancer) cells with lowest IC_{50} values were selected and further evaluated for *in-vivo* anticancer efficacy in MNU included breast cancer animal model.

For LD_{50} determination, compounds were administered to rats intraperitonially at various dose levels 50, 100, 200, 400 and 800 mg/kg in 10% DMSO and observed for first 2 hours and then at 6th and 24th hour for any toxic symptom.

After 24 hours, mortality rate of each group was observed. 100% death was recorded for the animals that received the dose of 800 mg/kg and >50% of animal death was observed at the dose of 400 mg/kg. LD_{50} values were calculated according to log-probit method **Table 5.8**.

Table 5.8: LD_{50} values of selected compounds

Compound code	**LD_{50} (mg/kg)**
MB-9	198.15 ± 7.47
MB-18	212.67 ± 5.31
MB-24	187.83 ± 6.45
MB-32	245.36 ± 4.57
Imatinib	589.7 ± 4.27

LD_{50} is the dose that killed 50% of animals of particular species, LD_{50} values were calculated according to log- probit method. All the values are expressed as mean ± SEM (n=3).

For determination of maximum tolerated dose (MTD), doses below the LD_{50} cut off values (50 mg/kg, 100 mg/kg and 200 mg/kg) were administered to the rats intraperitonially. At the dose of 50 mg/kg, i.p., neither mortality of animals nor any sign of toxicity or body weight loss was observed. Hence, the MTD of the test compounds was fixed as 50mg/kg and the doses below the MTD value (5-20 mg/kg BW.) were selected as screening doses for the evaluation of anticancer activity.

As shown in **Table 5.9** at the dose of 5 mg/kg in 10% DMSO intraperitonially, significant reduction of tumor growth, improvement in the haematological parameters and enhancement of the life span were observed in the groups treated with compound MB-9 and MB-24 whereas other compounds did not show significant reduction in tumor volume. Thus the compound MB-9 and MB-24 were further screened for in-vivo anticancer activity using three different doses (5, 10 and 20 mg/kg) to study the effect of concentration in tumor volume. **Figure 5.8** depicted the change in the tumor volume. As shown in the **Table 5.10**, compound MB-24 at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg, i.p. in 10% DMSO exhibited positive correlation of concentration with reduction in tumor volume and improve hematological profile along with increased life span, while in case of compound MB-24, the maximum effect was achieved at the dose of 10 mg/kg i.e. significant difference was not observed in tumor volume reduction at two different doses 10 and 20 mg/kg. Compound MB-24 showed more pronounced effect on reduction of WBC level, tumor volume and improvement in % increase in life span (% ILS) in comparison to MB-9. These results suggest that compound MB-24 is more active than MB-9 and needed some structural modification to obtain good anticancer candidate.

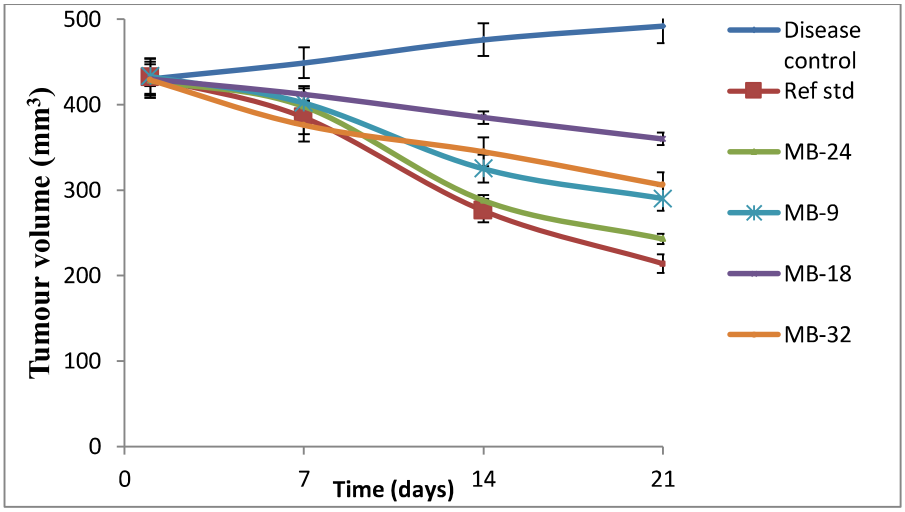


Figure 5.8: The change in the tumor volume after the treatment. Statically significance was determined applying *One-way ANOVA* followed by Tukey's test Experimental groups were compared with MNU control; (*) $P<0.05$ and (**) $P<0.01$ considered statistically significant. The results given are mean ± SEM (n=3).Ref std (Imatinib)

Table 5.9: Anticancer activity of selected compounds at the dose of 5mg/kg (i.p.) in MNU induced breast cancer bearing rats

Parameters at the dose of 5mg/kg body weight							
Group	Compound	RBC (million/mm^3)	WBC (10^3/mm^3)	Hb (g%)	MST[a]	%IL[b]	[c]Tumor Volume (mm^3)
I	Normal Saline	7.63 ±0.14	6.31 ±0.12	12.30 ± 0.13	-	-	-
II	MNU control	4.13 ± 0.36	13.23±0.45	8.2 ± 0.24	27	-	397 ± 1.13
III	Imatinib	6.32 ± 0.23**	8.21 ± 0.39**	12.30 ±0.50**	48.34	86.31	214±3.32**
IV	MB-9	5.89 ± 0.36**	7.31 ±0.23*	11.39 ±0.25*	36.35	25.43	290 ±1.98**
V	MB-18	4.56 ± 0.21**	6.67 ±0.32**	9.56 ±0.50**	29.21	35.57	360 ±2.21*
VI	MB-24	6.10 ± 0.11*	7.92 ± 0.63**	10.33 ±0.59**	39.34	54.63	243 ±3.67**
VII	MB-32	4.18 ± 0.58**	6.21 ±0.43**	9.73 ±0.36**	47.54	19.23	306±3.36*

Level of significance was determined applying *One-way ANOVA* followed by Tukey's test Experimental groups were compared with MNU control; (*) $P<0.05$ and (**) $P<0.01$ considered statistically significant. The results given are mean ± SEM (n=3).

[a]MST (Mean survival Time) = (Day of first death + Day of last death)/2.

[b]% ILS (Percentage increase in life span) = [(mean survival time of treated group/ mean survival time of control group)] – 1] ×100.

[c]Tumor volume = $a \times b^2/2$, a= largest diameter, b= shortest diameter of tumor.

Table 5.10: Anticancer effect of compound MB-9 and MB-24 at different doses on MNU induced breast cancer bearing rats

Parameters at doses 5mg, 10mg and 20mg/kg							
Group	**Compound code**	**RBC (million/mm^3)**	**WBC (10^3/mm^3)**	**Hb (g%)**	**MST**	**%ILS**	**Tumor volume**
I	Normal Saline	7.63 ±0.14	6.31 ±0.12	12.30 ± 0.13	-	-	-
II	MNU control	4.13 ± 0.36	13.23±0.45	8.2 ± 0.24	27	-	397 ± 1.13
III	Imatinib	6.32 ± 0.23^a	8.21 ± 0.39^a	12.30 ±0.50*	48.34	86.31	214±2.32^{a,b,c}
IV	MB-9(5mg/kg	5.89 ± 0.36^a	7.31 ±0.23^a	11.39 ±0.25*	36.35	25.43	290 ±1.98^a
V	MB-9 (10mg/kg)	6.21± 0.32^a	8.23 ±0.63^a	11.6 ±0.56*	37	51.94	241 ±2.65^a
VI	MB-9 (20mg/kg)	6.13 ±0.21^a	8.15±0.54^a	10.4 ±0.65*	38	53.32	238 ±2.84^{a,b}
VII	MB-24(5mg/kg)	6.10 ± 0.11^a	7.92 ± 0.63^a	10.33 ±0.59*	39.34	54.63	243 ±3.67^a
VIII	MB-24 (10mg/kg)	5.96 ±0.36^a	7.43±0.92^a	11.54 ±0.34*	38.76	65.17	224 ±2.54^{a,b,c}
IX	MB-24 (20mg/kg)	6.13 ±0.42^a	7.24 ± 0.37^a	12.18 ±0.54	49.54	74.23	216 ±3.43^{a,b,c}

The results given are mean ± SEM; n=3, Level of significance was checked by *One way ANOVA* followed by *Student NewmannKuels test.*
^{a}P<0.05 compared to MNU control, ^{b}P<0.05 compared to MB-9 (5mg/kg), ^{c}P<0.05 compared to MB-24 (5mg/kg).