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Original Article

DESIGN, SYNTHESIS, MOLECULAR DOCKING AND ANTIBACTERIAL EVALUATION OF NOVEL N-(6, 11-DIOXO-DIHYDRO-5H-BENZO [B] CARBAZOL-2YL) BENZAMIDE DERIVATIVES AS POTENT ANTIBACTERIAL AGENTS

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ABSTRACT

Objective: Heterocyclic quinone derivatives are important class of organic compounds both in biological and electrochemical applications. In this article our prime motivation is to develop novel series of heterocyclic quinone derivatives for antibacterial applications. Clinically isolated different Gram-negative and Gram-positive bacterial microorganisms were studied in this article and reported.

Methods: A novel series of N-(6,11-dioxo-dihydro-5H-benzo[b]carbazol-2yl) benzamide derivatives were synthesized by the Michael addition of 1,4-naphthoquinone and *para*-phenylene diamine. The derivatives of compound **1** were synthesized, subjecting it to benzolyation by a variety of acid chlorides. To understand the interaction of binding sites with bacterial protein receptor, the docking study was performed by glide program. *In vitro* antibacterial activity of the synthesized compounds was studied and the MIC value was calculated by the agar dilution method.

Results: The compound **2b** (0.4 µg/mL) exhibited good antibacterial activity against *staphylococcus aureus* than the standards sparfloxacin (4.87 µg/mL) and Norfloxacin (39.06 µg/mL) which was employed.

Conclusion: This investigation identified the potent antibacterial agents and these molecules will be subjected to further studies in our laboratory.

Keywords: Suzuki coupling, Palladium catalyst, 1,4-Naphthoquinone.

INTRODUCTION

In recent years infections caused by bacteria, resistant to multiple antibiotics has been an important problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococcus (VRE) are the most important infections caused by bacteria which have been found worldwide in hospitals [1]. Thus, the discovery of potent anti-bacterial agents is of great concern inside of multidrug resistance by Gram-positive and gram negative pathogens [2]. Quinones and heterocyclic quinones are large class of compounds with diverse biological activity. They are found to be very cheap and easily available having wide range of biological applications which includes electron transport and oxidative phosphorylation [3]. Heterocyclic quinones are biologically active [4] and heterocyclic aminoquinones have number of successive biological applications including anticancer [5], antibacterial [6-7], fungicidic [7-8], luciferase inhibition [9], antiproliferative [10] and tuberculostatic effects [11]. In addition, the heterocyclic naphthoquinone derivatives exhibit potent properties like electrochemical capacitance [12], electrochemical redox [13], electron mediator [14] and electron transfer [15] in many biological systems. They are also capable molecules of forming complexes with metals [16].In our previous reports [17-18] the novel quinones and heterocyclic quinones were studied for their fluorescent switching properties. In this report our interest is to study the antibacterial activity of novel N- (6,11-dioxo-dihydro- 5H-benzo [b] carbazol-2yl) benzamide derivatives which is not yet reported in literature, to the best of our knowledge**.** This present investigation deals with the clinically isolated different gram positive and gram negative bacteria against synthesized compounds and most of the tested compounds act as potent antibacterial agents. To understand the interactions of tested compounds at active sites of protein receptors the molecular docking studies were also preformed and reported in this article.

MATERIALS AND METHODS

Melting points (^{o}C, uncorrected) of all the synthesized compounds were checked in capillary tubes by using a digital melting point apparatus (Labtronics 110, India) and found uncorrected. All the analytical grade chemicals and solvents were purchased from Sigma–Aldrich and Merck, India. Progress and completion of all the reactions were monitored by thin layer chromatography (TLC silica gel 0.25 mm, 60 G F254 and eluting solvents were ethyl acetate: hexane 1:9). All the compounds were characterized by FT-IR spectrometer (IR Prestige-21, Shimadzu, Japan) using KBr pellets, ^{1}H NMR spectroscopy in DMSO-d_6 (500 MHz, Bruker), and ^{13}C NMR spectroscopy in DMSO- d_6 (125 MHz, Bruker) using tetramethyl silane (TMS) as internal standard. High resolution mass spectra (HRMS-EI) were measured by Electron Impact (EI) method (Jeol GC-Mate 2). Antibacterial studies were carried out by agar dilution method and the MICs were calculated for the tested compounds.

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

A mixture of 1,4-naphthoquinone (2 mmol, 0.316 g) and *para*- phenylene diamine (2 mmol, 0.216 g) was added to absolute ethanol (50 mL) and the mixture was refluxed for 10 h. The contents were cooled to room temperature and the reaction mixture was poured into water containing crushed ice and the precipitate formed was collected by vacuum filtration. The product was dried at 50 ºC and recrystalized from acetone. Black solid; (0.450 g, 80%); mp > 300 ºC; IR (KBr):1120, 1271, 1354, 1436, 1512, 1568, 1670, 3313; ^{1}H NMR (500 MHz, DMSO-d_6):5.23 (s, 1H), 5.86 (s, 1H), 6.62 (d, 2H, *J* = 8.4 Hz), 7.01 (d, 2H, *J* = 8.0 Hz,), 7.74-7.78 (t, 1H, *J* = 6.4 Hz), 7.82-7.86 (t, 1H, *J* = 6.4 Hz), 7.93 (d, 1H, *J* = 7.6 Hz), 8.03 (d, 1H, *J* = 7.6 Hz), 8.96 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6):100.2, 113.9, 114.0, 125.1, 125.2, 125.6, 126.4, 130.4, 132.2, 134.7, 146.9, 181.7, 181.8; HRMS (EI) *m/z:* Calcd for $C_{16}H_{12}N_2O_2$: 264.2786 Found: 264.2787.

General procedure for synthesis of N-(6,11-dioxo-dihydro-5H-benzo[b]carbazol 2yl) benzamide derivatives (2a-h)

Substituted acid chlorides (1 mmol) was added to a solution of **1** (0.264 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 **2a-h** which required no further purification.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)benzamide (2a)

Purple solid; Reaction time 25 minutes (0.345 g, 94%); mp > 300 ºC; IR (KBr): 1296, 1409, 1514, 1548, 1600, 1672, 3288 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.09 (s, 1H), 7.38 (d, 2H, *J* = 9.0 Hz), 7.47-7.56 (m, 4H), 7.59-7.62 (t, 1H, *J* = 6.0 Hz), 7.77-7.81 (t, 1H, *J* = 6.0 Hz), 7.85 (d, 2H, *J* = 6.0 Hz), 7.94-7.97 (t, 1H, *J* = 7.0 Hz), 7.98 (d, 1H, *J* = 7.2 Hz), 8.06 (d, 1H, *J* = 8.0 Hz) 9.23 (s, 1H), 10.36 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 102.1, 121.5, 124.5, 125.7, 126.5, 128.1, 128.8, 129.0, 131.2, 133.0, 135.3, 136.9, 146.7, 165.9, 182.8, 183.5; HRMS (EI) *m/z:* Calcd for $C_{23}H_{16}N_2O_3$: 368.3847 Found: 368.3873.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)-3-methylbenzamide (2b)

Purple solid; Reaction time 25 minutes (0.360 g, 94%); mp > 300 ºC; IR (KBr): 1190, 1234, 1296, 1354, 1408, 1512, 1546, 1598, 1666, 3300, 3365 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.41 (s, 3H), 6.09 (s, 1H), 7.37 (d, 2H, *J* = 8.5 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 7.75-7.80 (m, 4H), 7.84 (t, 2H, *J* = 8.0 Hz), 7.95 (d, 1H, *J* = 7.5 Hz), 8.13 (d, 1H, *J* = 7.5 Hz), 9.23 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 102.1, 121.5, 124.5, 125.2, 126.5, 128.5, 132.6, 135.3, 138.1, 147.0, 166.3, 182.3, 183.1; HRMS (EI) *m/z:* Calcd for $C_{24}H_{18}N_2O_3$: 382.4113 Found: 382.4112.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)-4-methylbenzamide (2c)

Purple solid; Reaction time 25 minutes (0.355g, 94%); mp > 300 ºC; IR (KBr): 1118, 1180, 1234, 1355, 1408, 1512, 1595, 1656, 1685, 3271 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 1.18 (s, 3H), 6.02 (s, 1H), 7.31-7.89 (m, 11H), 9.16 (d, 1H, *J* = 8.0 Hz), 10.20 (s, 1H), 12.76 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.3, 113.2, 117.0, 122.8, 124.3, 126.8, 132.5, 133.8, 134.8, 135.2, 136.8, 137.7, 138.0, 166.8, 178.2, 181.0; HRMS (EI) *m/z:* Calcd for $C_{24}H_{18}N_2O_3$: 382.4113 Found: 382.4113.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)-3-nitrobenzamide (2d)

Purple solid; Reaction time 25 minutes (0.390 g, 95%); mp > 300 ºC; IR (KBr): 1120, 1238, 1294, 1352, 1408, 1533, 1616, 1672, 3269 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.11 (s, 1H), 7.40 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 2H, *J* = 8.5 Hz), 7.94 (d, 2H, *J* = 8.0 Hz), 8.05 (d, 1H, *J* = 7.5 Hz), 8.33-8.50 (m, 4H), 8.61 (d, 1H, *J* = 8.0 Hz), 9.23 (s, 1H), 10.68 (s, 1H), 13.62 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 102.2, 121.7, 122.8, 124.1, 125.7, 126.5, 127.7, 130.6, 132.9, 133.1, 134.4, 135.3, 136.6, 146.6, 148.2, 163.7, 165.9, 182.0, 182.9; HRMS (EI) *m/z:* Calcd for $C_{23}H_{15}N_3O_5$: 413.3823 Found: 413.3822.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)-4-nitrobenzamide (2e)

Purple solid; Reaction time 25 minutes (0.385 g, 93%); mp > 300 ºC; IR (KBr): 1109, 1296, 1350, 1413, 1535, 1602, 1678, 3263 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.11 (s, 1H), 7.41 (d, 2H, *J* = 8.5 Hz), 7.79 (t, 1H, *J* = 8.2 Hz), 7.85 (d, 2H, *J* = 7.5 Hz), 7.95 (d, 2H, *J* = 6.8 Hz), 8.07 (d, 1H, *J* = 7.0 Hz), 8.16 (d, 2H, *J* = 8.0 Hz), 8.32 (d, 2H, *J* = 8.5 Hz), 8.38 (t, 1H, *J* = 7.5 Hz), 9.25 (s, 1H), 10.67 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 121.7, 124.0, 124.2, 124.5, 125.7, 129.7, 131.1, 136.8, 150.5, 160.5, 166.2, 180.0, 181.3; HRMS (EI) *m/z:* Calcd for $C_{23}H_{15}N_2O_5$: 413.3823 Found: 413.3822.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)-3,5-dinitrobenzamide (2f)

Purple solid; Reaction time 25 minutes (0.435 g, 95%); mp > 300 ºC; IR (KBr): 1080, 1122, 1159, 1242, 1294, 1344, 1409, 1516, 1543, 1668, 3078, 3267 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.13 (s, 1H), 7.14 (t, 1H, *J* = 8.0 Hz), 7.23 (t, 1H, *J* = 7.5 Hz), 7.34 (d, 1H, *J* = 7.5 Hz), 7.44 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 1H, *J* = 8.5 Hz), 7.86 (d, 2H, *J* = 8.8 Hz), 7.95 (d, 2H, *J* = 8.0 Hz), 8.08 (s, 1H), 9.26 (s, 1H), 10.96 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 102.3, 121.6, 122.5, 124.5, 125.7, 126.5, 128.5, 129.3, 130.8, 133.1, 135.9, 137.8, 148.2, 163.5, 182.0, 182.9; HRMS (EI) *m/z:* Calcd for $C_{23}H_{14}N_4O_7$: 458.3798 Found: 458.3798.

N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)acetamide (2g)

Purple solid; Reaction time 25 minutes (0.290 g, 95%); mp > 300 ºC; IR (KBr): 1122, 1269, 1294, 1357, 1409, 1523, 1566, 1606, 1683, 3194, 3309 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.06 (s, 3H), 6.03 (s, 1H), 7.30 (d, 2H, *J* = 8.5 Hz), 7.63 (d, 2H, *J* = 9.0 Hz), 7.77 (t, 1H, *J* = 7.5 Hz), 7.84 (t, 1H, *J* = 7.5 Hz), 7.94 (d, 1H, *J* = 7.5 Hz), 8.05 (d, 1H, *J* = 7.5 Hz), 9.17 (s, 1H), 9.26 (s, 1H), 10.04 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.7, 101.9, 120.1, 124.7, 125.7, 126.5, 130.9, 133.0, 135.3, 171.7, 183.0; HRMS (EI) *m/z:* Calcd for $C_{18}H_{14}N_2O_3$: 306.3153 Found: 306.3153.

2-chloro-N-(4-(1,4-dioxo-1,4-dihydronaphthalene-2ylamino)phenyl)acetamide (2h)

Purple solid; Reaction time 25 minutes (0.320 g, 94%); mp > 300 ºC; IR (KBr): 989, 1124, 1294, 1359, 1409, 1521, 1564, 1597, 1676, 3076, 3190 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 4.26 (s, 2H), 6.06 (s, 1H), 7.35 (d, 2H, *J* = 8.5 Hz), 7.66 (d, 2H, *J* = 8.5 Hz), 7.76 (t, 1H, *J* = 7.5 Hz), 7.84 (t, 1H, *J* = 7.5 Hz), 7.93 (d, 1H, *J* = 8.0 Hz), 8.05 (d, 1H, *J* = 7.5 Hz), 9.20 (s, 1H), 10.40 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.01, 102.1, 120.6, 124.7, 125.7, 126.5, 126.5, 130.8, 133.0, 133.1, 134.1, 135.3, 136.1, 146.7, 165.0, 182.0, 182.8; HRMS (EI) *m/z:* Calcd for $C_{18}H_{13}ClN_2O_3$: 340.7604 Found: 340.7604.

General Procedure for synthesis of N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)benzamide derivatives (3a-h)

Mixture of **2a-h** (0.5 mmol) in glacial acetic acid (60 mL) and palladium (II) acetate (0.112 g, 0.5 mmol) were refluxed for 2 h and the reaction mixture was cooled at room temperature and poured into ice cold water. The precipitate was filtered, dried at 60 ºC and crystallized from acetone to give **3a-h**.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)benzamide (3a)

Yellow solid; Reaction time 2 hours (0.265 g, 73%); mp > 300 ºC; IR (KBr): 1010, 1240, 1271, 1375, 1481, 1587, 1647, 3277 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 7.45-7.61 (m, 5H), 7.80-7.89 (m, 4H), 8.02 (d, 2H, *J* = 7.0 Hz), 8.75 (s, 1H), 10.43 (s, 1H), 13.09 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 113.4, 114.2, 122.2, 124.6, 126.4, 128.1, 132.0, 133.1, 134.6, 135.4, 136.3, 137.9, 165.9, 177.8, 180.6; HRMS (EI) *m/z:* Calcd for $C_{23}H_{14}N_2O_3$: 366.3688 Found: 366.3687.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)-3-methylbenzamide (3b)

Yellow solid; Reaction time 2 hours (0.280 g, 74%); mp > 300 ºC; IR (KBr): 1012, 1238, 1273, 1321, 1377, 1431, 1481, 1535, 1591, 1645, 1668, 2920, 3277 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.43 (s, 3H), 7.43 (d, 2H, *J* = 7.0 Hz), 7.59 (d, 1H, *J* = 8.0 Hz), 7.81 (d, 1H, *J* = 7.5 Hz), 7.83 (d, 1H, *J* = 6.0 Hz), 7.89 (t, 1H, *J* = 8.0 Hz), 8.10 (d, 1H, *J* = 7.0 Hz), 7.59 (d, 1H, *J* = 8.0 Hz), 8.13 (d, 1H, *J* = 8.0 Hz), 8.74 (s, 1H), 10.37 (s, 1H), 13.08 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 21.4, 113.3, 114.2, 117.8, 122.2, 124.6, 125.3, 126.4, 128.6, 132.5, 133.1, 134.6, 135.4, 136.3, 137.9, 138.1, 166.0, 177.8, 180.6; HRMS (EI) *m/z:* Calcd for $C_{24}H_{16}N_2O_3$: 380.3954 Found: 380.3954.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)-4-methylbenzamide (3c)

Yellow solid; Reaction time 2 hours (0.278g, 74%); mp > 300 ºC; IR (KBr): 1010, 1273, 1323, 1483, 1527, 1589, 1641, 1668, 2358, 2848, 2918, 3267 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 1.23 (s, 3H), 7.35 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 1H, *J* = 8.5 Hz) 7.81-7.89 (m, 3H), 7.94 (d, 2H, *J* = 8.0 Hz), 8.10 (d, 1H, *J* = 7.5 Hz), 8.15 (d, 1H, *J* = 7.5 Hz), 8.74 (s, 1H), 10.33 (s, 1H), 13.08 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 21.3, 114.7, 115.3, 117.3, 121.3, 124.7, 128.8, 133.2, 134.0, 135.0, 136.3, 137.5, 138.8, 166.1, 177.0, 178.3; HRMS (EI) *m/z:* Calcd for $C_{24}H_{16}N_2O_3$: 380.3954 Found: 380.3953.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)-3-nitrobenzamide (3d)

Yellow solid; Reaction time 2 hours (0.300 g, 72%); mp > 300 ºC; IR (KBr): 1010, 1240, 1271, 1327, 1485, 1527, 1591, 1668, 2918, 3205 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 7.61 (d, 1H, *J* = 6.8 Hz), 7.69 (d,

1H, *J* = 7.5 Hz), 7.83-7.99 (m, 7H), 8.74 (s, 1H), 8.87 (s, 1H), 10.75 (s, 1H), 13.11 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 105.3, 110.4, 113.6, 118.1, 122.1, 126.5, 130.6, 134.6, 135.7, 136.7, 148.2, 163.7, 177.8, 178.3; HRMS (EI) *m/z:* Calcd for $C_{23}H_{13}N_3O_5$: 411.3664 Found: 411.3664.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)-4-nitrobenzamide (3e)

Yellow solid; Reaction time 2 hours (0.305 g, 74%); mp > 300 ºC; IR (KBr): 1010, 1273, 1346, 1523, 1595, 1674, 2918, 3263 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 7.75 (d, 2H, *J* = 8.0 Hz), 7.73 (d, 2H, *J* = 8.0 Hz), 8.05-8.43 (m, 7H), 10.74 (s, 1H), 13.13 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 99.8, 124.0, 127.4, 129.7, 141.2, 153.8, 163.2, 180.1, 181.7; HRMS (EI) *m/z:* Calcd for $C_{23}H_{13}N_3O_5$: 411.3664 Found: 411.3664.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)-3,5-dinitrobenzamide (3f)

Yellow solid; Reaction time 2 hours (0.340 g, 75%); mp > 300 ºC; IR (KBr): 1016, 1155, 1246, 1330, 1489, 1541, 1587, 1666, 2918, 3084, 3203, 3390 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 7.18 (d, 1H, *J* = 5.8 Hz), 7.36 (d, 1H, *J* = 6.2 Hz), 7.76-8.02 (m, 7H), 8.72 (s, 1H), 11.01 (s, 1H), 13.15 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 100.18, 102.7, 128.5, 134.8, 138.4, 148.6, 169.5, 180.2, 181.7; HRMS (EI) *m/z):* Calcd for $C_{23}H_{12}N_4O_7$: 456.3639 Found: 456.3639.

N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)acetamide (3g)

Yellow solid; Reaction time 2 hours (0.230 g, 75%); mp > 300 ºC; IR (KBr): 1008, 1240, 1273, 1369, 1516, 1589, 1647, 2926, 3442 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 1.23 (s, 3H), 7.51 (d, 1H, *J* = 8.5 Hz), 7.64 (d, 1H, *J* = 8.0 Hz), 7.71 (s, 1H), 7.80-7.90 (m, 4H), 10.09 (s, 1H), 13.02 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 24.4, 114.4, 126.5, 133.2, 134.6, 168.9, 180.7, 183.0; HRMS (EI) *m/z:* Calcd for $C_{18}H_{12}N_2O_3$: 304.2994 Found: 304.2994.

2-chloro-N-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazol-2-yl)acetamide (3h)

Yellow solid; Reaction time 2 hours (0.250 g, 74%); mp > 300 ºC; IR (KBr): 1006, 1273, 1375, 1531, 1587, 1672, 3275, 3739 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 2.09 (s, 2H), 7.51 (d, 1H, *J* = 6.5 Hz), 7.63 (d, 1H, *J* = 7.5 Hz), 7.80-7.90 (m, 2H), 8.09-8.16 (m, 2H), 10.08 (s, 1H), 10.47 (s, 1H), 13.08 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 69.0, 101.3, 102.6, 102.7, 103.1, 104.7, 105.8, 110.3, 113.8, 114.1, 118.2, 121.3, 126.8, 130.2, 132.0, 135.8, 137.8, 141.2, 163.2, 171.4, 178.2; HRMS (EI) *m/z:* Calcd for $C_{18}H_{11}ClN_2O_3$: 338.7445 Found: 338.7445.

Molecular docking studies

To understand the interaction of all the synthesized molecules (**1, 2a-h, 3a-h**) with *Bacillus substillis*, the crystal structure of YmaH from Bacillus subtillis [21] were downloaded from protein data bank and the molecular docking studies were performed using the GLIDE program [22] (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. YmaH (PDB ID: 3HSB) were selected for docking studies as a reference sample and was prepared for docking through protein preparation wizard. Structures of **1, 2a-h, 3a-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.

***In vitro* Antibacterial activity**

All the synthesized compounds were studied for their antibacterial activity against clinically isolated two Gram-positive bacteria (*Bacillus subtillisn and Klebsiella Pneumoniae*) and five Gram-negative bacilli (*Staphylcoccous aureus, Escherichia coli, Proteus vulgaris, Salmonella typhi, Pseudomonas aureus*) using conventional agar-dilution method [23-24]. The minimum inhibitory concentrations (MICs) values were calculated by comparison to Sparfloxacin and Norfloxacin as the reference bacterial drugs and they are presented in Table 1. All the cultures were prepared by Muller Hinton agar and the turbidity of all the bacterial cultures was adjusted to 0.5 McFarland Standard by preparing bacterial suspension of 3–5 well-isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted 1000-fold to get an inoculums size of 1.5 X 10^5 CFU/The synthesized compounds and standard bacterial drugs (50 mg) were dissolved in Dimethyl formamide (DMF) (0.5 mL) and the solution was diluted with water (4.5 mL) to get a stock solution of 10,000 mg/L of each compound. Further progressive double dilution with Muller–Hinton broth was performed to obtain the required concentrations of 2500-0.7 µg/mL [25]. To ensure that the solvent had no effect on the bacterial growth, a control test was performed with a test medium supplemented with DMF at the same dilutions as used in the experiment.

In each micro well inoculated with 75 µL of the serial dilutions, 75 µL of the bacterial suspension was added in a series of 12 micro wells. Incubation of the cultures overnight at 37 ºC was done and the growth measured. The MICs of the test compounds and the standard control drugs are tabulated in Table 1.

RESULTS AND DISCUSSION

Chemistry

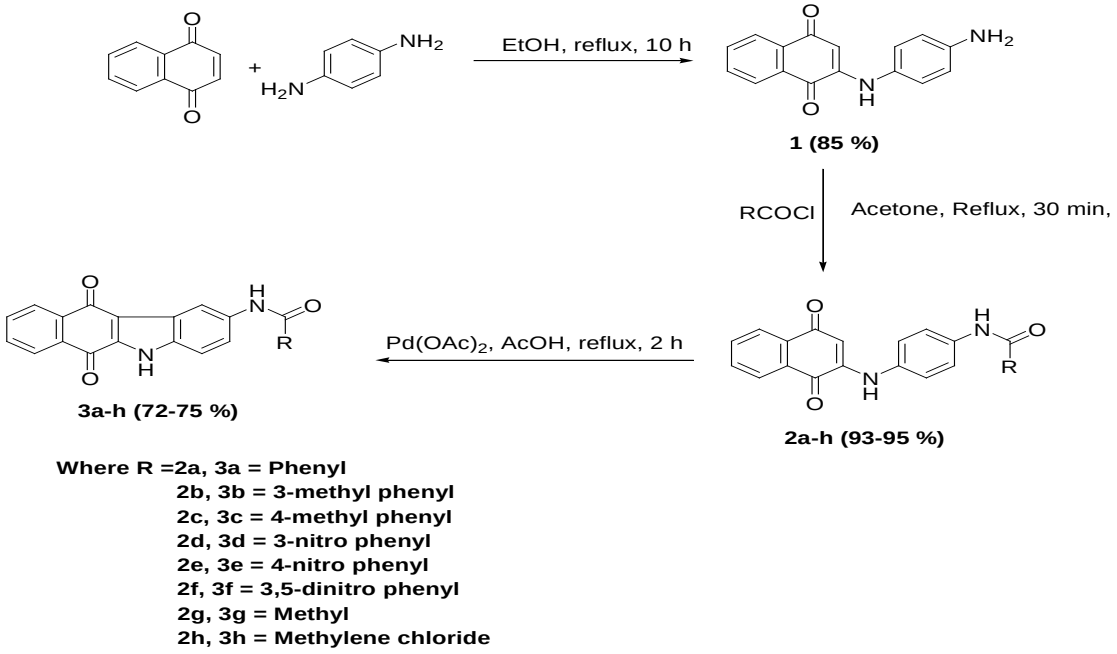
In our previous report, simple "off-on-off" chemical and electrochemical fluorescent switches were successfully demonstrated [18]. The compound (**1**) was synthesized by the nucleophilic amino addition reaction of equal mole concentrations of 1,4-naphthoquinone and *para*-phenylene diamine in the presence of absolute ethanol. The mixture was refluxed for 10 h and the yield obtained was 85%. In a previous report [19] the same compound (**1**) was synthesized by amino substitution in the presence of absolute ethanol at room temperature. The product was obtained after 96 h of stirring and the percentage of yield compound **1** was not given. In another report [20] the same reaction was carried out in the presence of water-glacial acetic acid mixture under reflux condition. The yield reported was between 49-62%. In one of our previous study we reported [17] the nucleophilic amino addition and substitution reactions with 1,4-quinone moiety in the presence of ethanol, water and solvent free microwave system. Based on the above literature we attempted the amino substitution reaction with 1,4-naphthoquinone in the presence of water as solvent and the reaction was not successful. A number of inseparable compounds were formed and the yield was low (25%) compared to ethanol mediated amino addition. In the second step, the intra molecular carbon-carbon bond linkage was carried out by palladium (II) acetate in the presence of glacial acetic acid and the product yield was 72-75%. The percentage of yield for compounds (**3a-h**) is comparatively very good. In the reported work [19] no information about the product yield was given and in another report [20] the yields were 18-62% for the palladium catalyzed reaction.

Biology

***In vitro* antibacterial studies of quinone derivatives**

All the synthesized compounds were tested against two Gram-positive and five Gram-negative bacteria's. All the compounds (**1, 2a-h, 3a-h**) exhibited good bacterial activity against gram positive bacteria of *Klebsiella Pneumoniae* than the standard drugs used (Sparfloxacin and Norfloxacin). Compound **3f** exhibits good activity against most of the gram positive and gram negative microorganisms due to the presence of the two nitro groups at the third and fifth position of the aromatic system of the benzoyl unit. Compound **2f** exhibits better activity against *Escherichia coli* (98 µg/mL) than Sparfloxacin (156.3 µg/mL) and Norfloxacin (625 µg/mL). Compound **1** (1.2 µg/mL) exhibits better activity against *Proteus vulgaris* than the standard drug Sparfloxacin (4.8 µg/mL). Compound **3c** (227 µg/mL) exhibits better bacterial activity against *Salmonella typhi* than Sparfloxacin (2500 µg/mL) and Norfloxacin (627 µg/mL). Compound **3a** (32 µg/mL), **3b** (27 µg/mL), **3c** (23 µg/mL) exhibits better antibacterial activity against Pseudomonas aureus than the standards of Sparfloxacin (156.3 µg/mL) and

Norfloxacin (39.06 µg/mL). Compounds **2b** (0.4 µg/mL) and **2f** (0.4 µg/mL) exhibited very good antibacterial activity due to the presence of methyl and nitro functional groups, among all the molecules synthesized against *Staphylococcus aureus* than Sparfloxacin (4.87 µg/mL) and Norfloxacin (39.06 µg/mL). Standard drug Norfloxacin could not exhibit any activity against *Bacillus subtillis* and *Proteus vulgaris* microorganisms. Compound **2g** did not exhibit any inhibition against *Bacillus subtillis, Escherichia coli, Proteus vulgaris* and compound **3d** did not exhibited any inhibition against *Escherichia coli.*



Scheme 1: The synthesis of 2-(4-aminophenylamino)naphthalene-1,4-dione (1), N-(6,11-dioxo-dihydro-5H-benzo[b]carbazol 2yl)benzamide derivatives (2a-h) and title compounds of carbazole-6,11-dione derivatives (3a-h).

Table 1: *In vitro* antibacterial activity of synthesized compounds against Gram-positive and Gram-negative bacteria (MICs in µg/mL) Values were the means of three replicates ± SD.

Compounds	MIC (µg/mL)						
	B. subtilis	***S. aureus***	***E. coli***	***P.vulgaris***	***S. typhi***	***P.aureus***	***K. Pneumoninae***
1	33.9 ± 0.52	29 ± 0.25	621 ± 0.29	**1.2** ± 0.10	431 ± 0.52	113 ± 0.88	**214** ± 0.81
2a	52.8 ± 0.23	17 ± 0.28	**98** ± 0.19	12 ± 0.23	521 ± 0.12	63.5 ± 0.85	387 ± 0.65
2b	52 ± 0.83	**0.4** ± 0.12	216 ± 0. 23	21 ± 0.59	523 ± 0.36	145 ± 0.63	287 ± 0.33
2c	26.1 ± 0.22	**2.6** ± 0.23	214 ± 0.92	18 ± 0.56	432 ± 0.89	136 ± 0.92	298 ± 0.92
2d	29.3 ± 0.12	523 ± 0.89	214 ± 0.89	453 ± 0.81	752 ± 0.53	57 ± 0.93	278 ± 0.81
2e	31.7 ± 0.33	28.6 ± 0.32	213 ± 0.22	18 ± 0.72	654 ± 0.72	122 ± 0.33	342 ± 0.93
2f	52 ± 0.11	**0.4** ± 0.18	216 ± 0.36	21 ± 0.21	523 ± 0.36	145 ± 1.02	287 ± 0.33
2g	*	36 ± 0.59	*	*	523 ± 0.72	54 ± 0.99	521 ± 0.92
2h	31.2 ± 0.82	41 ± 0.93	523 ± 0.82	19 ± 0.92	346 ± 0.36	132 ± 0.33	647 ± 0.25
3a	524 ± 0.36	19 ± 0.36	348 ± 0.52	523 ± 1.23	892 ± 0.80	**32** ± 0.82	432 ± 1.23
3b	457 ± 0.87	14.53 ± 0.91	321.5 ± 0.23	568 ± 1.32	1052 ± 1.28	**27** ± 0.91	654 ± 1.02
3c	**8.8** ± 0.12	12.4 ± 0.25	391 ± 0.23	47 ± 1.09	**227** ± 0.82	**23** ± 1.22	897 ± 0.92
3d	21.3 ± 022	25 ± 0.82	*	13 ± 0.93	324 ± 0.69	91 ± 1.39	826 ± 0.24
3e	53 ± 0.33	17 ± 0.36	356 ± 0.82	27 ± 0.56	500 ± 0.36	142 ± 0.93	973 ± 0.78
3f	**6** ± 0.09	23.5 ± 0.25	326 ± 1.09	523 ± 0.23	563 ± 0.85	14 ± 0.23	825 ± 0.82
3g	94 ± 0.17	6.8 ± 0.83	489 ± 0.23	32 ± 0.89	765 ± 0.22	124 ± 0.89	956 ± 0.71
3h	83.1 ± 0.41	**4.5** ± 0.89	567 ± 0.29	321 ± 0.56	523 ± 1.09	83 ± 0.99	2000 ± 0.23
Sparfloxacin[a]	9.76 ± 0.52	4.87 ± 0.25	156.3 ± 0.89	4.8 ± 0.27	2500 ± 0.99	156 ± 0.39	2500 ± 1.22
Norfloxacin[a]	*	39.06 ± 0.23	625 ± 1.20	*	627 ± 0.52	39.06 ± 1.23	<1.2 ± 0.92

* No inhibition observed [a] Standard antibacterial drugs Lower MIC values indicates that higher antimicrobial activity Bold letters indicates better activity against microorganisms

Molecular docking studies of quinone derivatives

To understand the interaction of bacterial protein receptor with synthesized molecules (**1, 2a-h, 3a-h**) the crystal structure of YmaH from Bacillus subtillis was downloaded from protein data bank and studied with the glide program. The entire glide, E model scores and hydrogen bonds interactions are compared with the MICs of Bacillus subtillis for the tested compounds a are presented in table 2. The use of glide and E model scores for ranking the different derivatives within a series is always not dependable. The molecular docking and *in vitro* antibacterial study results show that the glide scores and MIC values of the synthesized compounds do not have any correlation. The glide scores are mainly used to identify the active and inactive compounds. In addition, glide is primarily concerned with generating an accurate pose for each ligand and enrichment (the separation of actives from inactives) (See figures 1-4) [22, 26].

Table 2: Molecular docking studies of ten analogues taken for study with *Bacillus subtillis* (PDB ID: 3HSB)

Compounds	Glide score	E model score (kcal/mol)	Molecular docking	
			No. of Hydrogen bonds interactions	MICs of BS (µg/mL)
1	-4.92	-34.71	2 (ASP 269, HIS 180)	33.9
2a	-5.33	-57.97	1 (LEU 142)	52.8
2b	-4.47	-60.14	2 (HIE 268, LYS 179)	52
2c	-5.86	-63.79	1 (GLN 63)	26.1
2d	-5.05	-63.29	1 (ASP 269)	29.3
2e	-5.12	-64.62	2 (ASP 269, LYS 179)	31.7
2f	-3.86	-75.11	Hydrophobic interaction	52
2g	-5.16	-49.42	1 (ASP 269)	*
2h	-5.78	-60.78	2 (GLN 63, GLN 208)	31.2
3a	-3.26	-63.54	1 (ASN 273)	524
3b	-3.96	-70.62	1 (ASN 273)	457
3c	-3.96	-66.01	1 (ASN 273)	8.8
3d	-3.16	-72.76	1 (ASN 273)	21.3
3e	-1.01	-69.72	1 (ASN 273)	53
3f	**-4.24**	**-72.39**	**2 (ASP 274, TRP 58)**	**6**
3g	-5.77	-44.69	1 (GLN 208)	94
3h	-4.87	-52.74	1 (GLN 63)	83.1
Sparfloxacin	-	-	-	9.76
Norfloxacin	-	-	-	*

-Docking studies not carried out * No inhibition observed Bold letters indicates better activity glide score against *Bacillus subtillis* (BS)

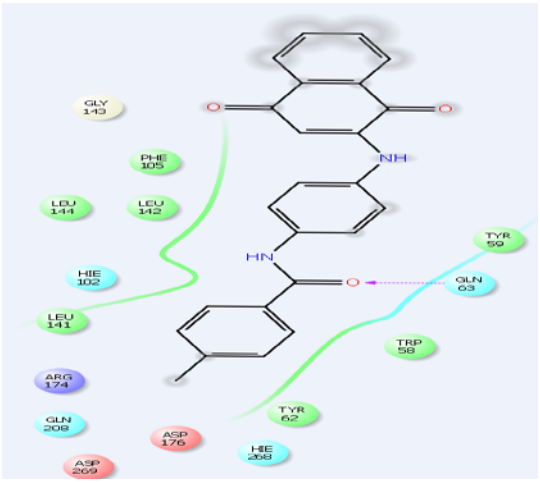


Fig. 1: Docking model structure of compound 2c respectively into the YmaH binding pocket.

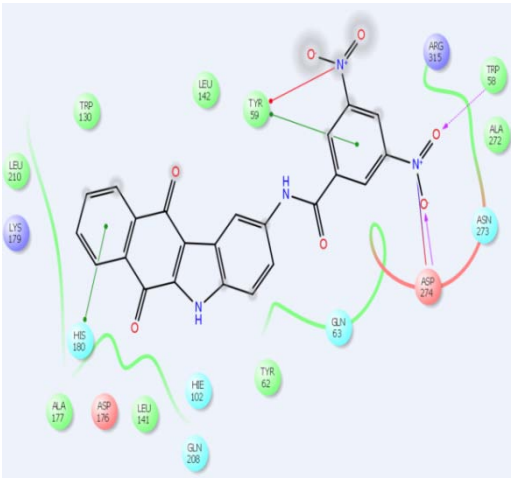


Fig. 3: Docking model structure of compound 3f respectively into the YmaH binding pocket.

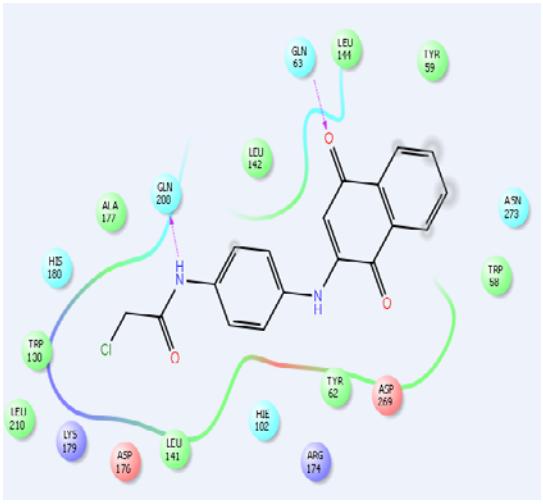


Fig. 2: Docking model structure of compound 2h respectively into the YmaH binding pocket.

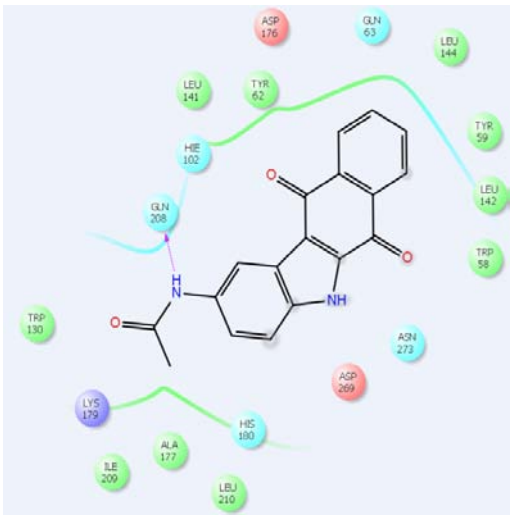


Fig. 4: Docking model structure of compound 3g respectively into the YmaH binding pocket.

CONCLUSION

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

CONFLICT OF INTEREST

Declared None

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