Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 139 (2015) 477–487

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Synthesis, molecular docking and biological evaluation of novel 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a] phenothiazin-5-one derivatives

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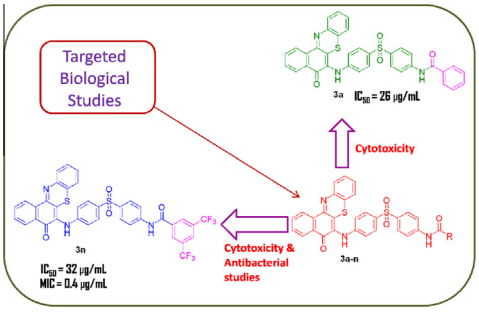
HIGHLIGHTS

- The compound **1** synthesized by a green route using water as solvent.
- The final compounds (**3a–n**) were obtained in better yield of about 93–94%.
- Most of the compounds exhibited better antibacterial properties.
- Compound **3a** and **3n** exhibits better cytotoxicity against cervical cancer cell line (SiHa).
- Compound **3n** exhibited better antibacterial property in a concentration of 0.4 μg/mL against *Bacillus subtilis*.

GRAPHICAL ABSTRACT

Equal mole concentrations of starting materials gave the final products in multistep reactions. The compound **3a** (IC_{50} = 26 μg/mL) act as a better anticancer agent and compound **3n** (IC_{50} = 32 μg/mL & MIC = 0.4 μg/mL) plays a dual role as better anticancer and antibacterial agent.



ARTICLE INFO

Article history:
Received 10 May 2014
Received in revised form 19 July 2014
Accepted 9 December 2014
Available online 19 December 2014

Keywords:
Solfones
Water as solvent
SiHa
Anticancer

ABSTRACT

A novel series of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives have been synthesized and examined for their *in vitro* antibacterial activity against a panel of Gram-positive and Gram-negative bacteria. Among these, N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl)phenyl)-3,5-bis(trifluoromethyl)benzamide (**3n**) (0.4 μg/mL) and 4-ethyl-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl)phenyl)benzamide (**3l**) (0.6 μg/mL) systems exhibited a potent inhibitory activity against Gram-positive organism *Bacillus subtilis*, when compare to the other synthesized compounds. Sparfloxacin (9.76 μg/mL), Norfloxacin (no activity) were employed as the standard drugs. An evaluation of the cytotoxicity of the title compounds (**1**, **2**, **3a–n**) revealed that they displayed low toxicity (26–115 mg/L) against cervical cancer cell line (SiHa). The results of these studies suggest that, phenothiazin-5-one derivatives are interesting binding agents for the development of new Gram-positive and Gram-negative antibacterial agents. To understand the interactions with protein receptors, docking simulation was done with crystal structures of *B. subtilis* (YmaH) and histone deacetylase (HDAC8) to determine the probable binding conformation.

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http://dx.doi.org/10.1016/j.saa.2014.12.036
1386-1425/

Introduction

The effect of bacterial infection still remains an important and serious problem due to a combination of factors including the emergence of infectious diseases and also due to the increase of multi-drug resistant microbial pathogens [1]. This problem has wide interests and antibacterial agents led us to the development of new antibacterial drugs [2]. Heterocyclic quinones, containing nitrogen atoms, are known to possess potent biological activity toward viral [3], molluscidal [4], malarial [5], leishmanial [6], antitumor [7,8], bacterial and fungal diseases [9–12], due to their hydroquinone–quinone redox potentials [13,14].

The aim of medicinal chemistry programs in the present scenario is to discover new heterocyclic quinones endowed with antibacterial activities. Therefore we developed an interest in this area and synthesized a series of novel 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives possessing one nitrogen atom in the heterocyclic ring (**2**, **3a–n**). The presence of aryl, alkyl group or nitrogen atom is an important factor to influence the antibacterial activities.

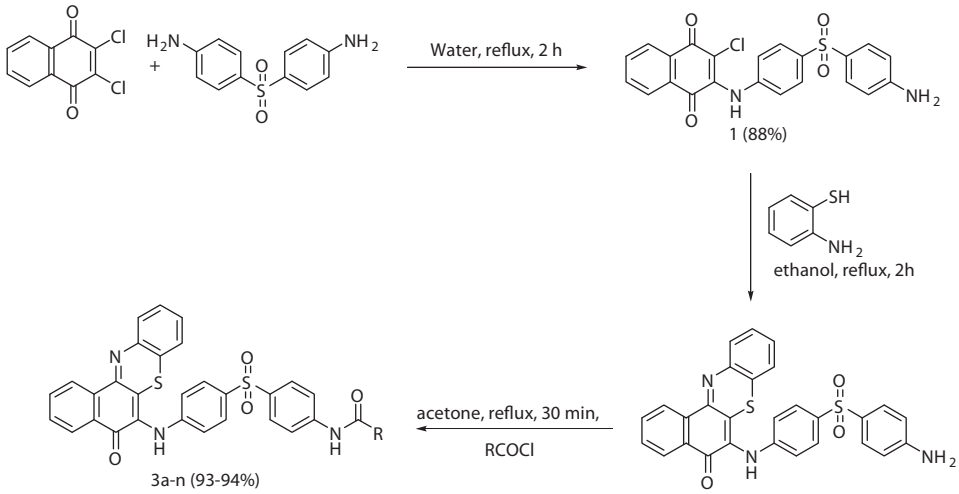
The cervical cancer is a second most common cancer in females has been identified worldwide and 12,200 new females suffer from cervical cancer and about 4210 deaths are attributed to cervical cancer in the United States alone in a year [16,29,30]. Therefore, our efforts are to develop the novel and effective therapeutic agents for cancer treatment, selective HDAC inhibitors have to design and synthesis has become one of our major goals. In this connection we synthesized a new series of novel 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives and studied their cytotoxicity against human cervical cancer cell line (SiHa) and the molecular docking studies of all the synthesized compounds were carried out with histone deacetylase (HDAC8) protein receptor and reported.

In our previous reports [15,16] the heterocyclic naphthoquinone derivatives with carbazole-6,11-dione moiety was studied for their molecular fluorescent switching properties and cytotoxicity against cervical cancer cell line (SiHa). In this report, we attempt to synthesize a series of novel phenothiazin-5-one moiety and their antibacterial and cytotoxicity behavior. But in comparison to carbazole-6,11-dione derivatives, the phenothiazin-5-one derivatives are very effective against different Gram-negative and Gram-positive bacteria but of lesser effect against cancer cell line (SiHa). Based on our results, we infer that a phenothiazin-5-one moiety can act as very good antibacterial agents against different bacterial pathogens.

Experimental

Materials and methods

Melting points (°C, uncorrected) of the synthesized compounds were checked in the open capillary tubes using a digital auto melting point apparatus (Labtronics 110, India) and found uncorrected. All the chemicals and solvents were purchased from Sigma–Aldrich, Merck and Himedia, India. Purity of all the products was checked by thin layer chromatography on a TLC silica gel 60 F254 using eluting solvents such as ethyl acetate and hexane (1:1). The synthesized compounds were purified by column chromatography using column silica gel 100–200 mesh (ethyl acetate:hexane 1:2). All the compounds were characterized employing a FT-IR spectrometer (IR 8400, Shimadzu, Japan) using KBr pellets. ^{1}H NMR spectroscopy in DMSO-d_6 (500 MHz, Bruker),



Where R = 3a = Phenyl
3b = 3-methyl phenyl
3c = 4-methyl phenyl
3d = 3-nitro phenyl
3e = 4-nitro phenyl
3f = 3,4-dimethoxy phenyl
3g = Methyl
3h = Methylene chloride
3i = 4-trifluoromethoxy phenyl
3j = 4-pentyl phenyl
3k = 4-methyl-3-nitro phenyl
3l = 4-ethyl phenyl
3m = 4-heptyl phenyl
3n = 3,5-bis-trifluoro methyl phenyl

Scheme 1. Synthetic scheme for 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives (**3a–n**).

^{13}C NMR spectroscopy in DMSO-d_6 (125 MHz, Bruker) using tetramethylsilane (TMS) as internal standard were also carried out. The Coupling constant (*J* values) is reported in Hz. High-resolution Mass spectra (HRMS-EI) was measured by Electron ionization (EI) method (Jeol GC-Mate 2). Molecular docking studies of all the synthesized compounds were accomplished by GLIDE program (version 8.5, Schrodinger, LLC, New York, 2010) and the entire glide scores are reported in kcal/mol. *In vitro* cytotoxicity of all the compounds against cervical cancer cell line (SiHa) was studied by cell viability assay method. The *in vitro* antibacterial study was carried out by agar dilution method and the MIC values were calculated for the tested compounds.

*Synthesis of 2-(4-(4-aminophenylsulfonyl) phenylamino)-3-chloronaphthalene-1,4 dione (**1**) [17]*

A mixture of 2,3-dichloro-1,4-naphthoquinone (2.270 g, 10 mmol) and 4-aminophenyl sulfone (2.048 g, 10 mmol) was added to distilled water (800 mL) and refluxed for 2 h. The reaction mixture was cool to room temperature. The red precipitate formed, separated by filtration, washed with hot water (200 mL), dried at 80 °C, and crystallized from 95% ethyl alcohol to give compound **1** (3.850 g, 88%) as red crystals; mp-239.5–240.5 °C [18]; IR (KBr): 1259, 1317, 1516, 1585, 1627, 1676, 1699, 2312, 3286, 3707 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 6.12 (s, 2H), 6.60 (d, 2H, *J* = 5.2 Hz), 7.17 (d, 2H, *J* = 4.8 Hz), 7.52 (d, 2H, *J* = 6.8 Hz), 7.70 (d, 2H, *J* = 6.8 Hz), 7.80 (t, 1H, *J* = 7.2 Hz), 7.85 (t, 1H, *J* = 7.0 Hz), 8.02 (t, 2H, *J* = 8.0 Hz), 9.50 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 112.9, 119.0, 122.1, 125.9, 126.1, 126.5, 126.6, 129.1, 130.4, 131.6, 133.4, 134.6, 137.0, 142.5, 143.1, 153.3, 176.9, 179.7; HRMS (EI) *m/z:* Calcd for $C_{22}H_{15}ClN_2O_4S$: 438.8835 found: 438.8832.

*Synthesis of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one (**2**)*

Compound **1** (2.194 g, 5 mmol) were added to the solution to 2-amino thiophenol (0.625 g, 5 mmol) in 95% of ethyl alcohol (500 mL) and the reaction mixture refluxed for 2 h. The reaction mixture was cooled to room temperature and poured into crushed ice, the dark brown precipitate formed was filtered by *vacuum* filtration and the precipitate was washed with distilled water (200 mL), dried at 80 °C, and crystallized from dried acetone to give compound **2** (2.100 g, 82%) as dark brown crystals; mp > 300 °C; IR (KBr): 889,1012, 1105, 1141, 1303, 1500, 1593, 1624, 3269, 3383 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 6.08 (s, 2H), 6.59 (d, 2H, *J* = 8.5 Hz), 6.78 (d, 2H, *J* = 8.5 Hz), 7.37 (t, 1H, *J* = 6.5 Hz), 7.48 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 2H, *J* = 8.5 Hz), 7.77 (t, 1H, *J* = 7.5 Hz), 7.84 (t, 1H, *J* = 8.0 Hz), 7.91 (t, 1H, *J* = 8.0 Hz), 7.99 (d, 1H, *J* = 8.0 Hz), 8.21 (d, 1H, *J* = 8.0 Hz), 8.57 (d, 1H, *J* = 8.0 Hz), 8.76 (s, 1H), 8.88 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 113.3, 115.6, 123.9, 124.3, 125.8, 126.0, 126.2, 127.5, 128.2, 128.4, 128.7, 129.3, 130.4, 130.6, 132.0, 132.1, 132.5, 132.8, 133.1, 134.5, 136.5, 138.3, 145.1, 146.6, 150.0, 153.5, 176.6; HRMS (EI) *m/z:* Calcd for $C_{28}H_{19}N_3O_3S_2$: 509.5987 found: 509.5987.

*General procedure for synthesis of 6-(4-(4-aminophenylsulfonyl)-phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives (**3a–n**)*

Substituted acid chlorides (1 mmol) were added to a solution of compound **2** (0.509 g, 1 mmol) in acetone (100 mL). After refluxing for 30 min, the reaction mixture filtered and concentrated *in vacuo* to give pure samples of **3a–n** which required no further purification.

*N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl)-phenyl)benzamide (**3a**)*

Brown solid; reaction time 30 min (0.575 g, 94%); mp > 300 °C; IR (KBr): 891, 1105, 1155, 1251, 1309, 1400, 1500, 1523, 1593, 1618, 1674, 3292 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 6.82 (d, 2H, *J* = 8.0 Hz), 7.49–7.64 (m, 10H), 7.69 (d, 2H, *J* = 8.5 Hz), 7.78 (d, 1H, *J* = 6.5 Hz), 7.85 (d, 2H, *J* = 8.2 Hz), 7.92 (d, 2H, *J* = 8.2 Hz), 8.00 (d, 1H, *J* = 8.0 Hz)8.21 (d, 1H, *J* = 6.8 Hz), 8.46 (t, 1H, *J* = 6.5 Hz), 10.64 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 115.7, 120.6, 123.8, 125.8, 126.0, 126.3, 127.1, 128.0, 128.3, 128.4, 128.8, 128.9, 129.0, 129.2, 129.7, 130.7, 131.2, 132.1, 132.4, 132.6, 133.2, 133.3, 134.5, 134.8, 137.0, 138.3, 143.8, 145.1, 147.6, 166.5, 167.7, 176.5; HRMS (EI) *m/z:* Calcd for $C_{35}H_{23}N_3O_4S_2$: 613.7048 found: 613.7048.

*3-methyl-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)-phenylsulfonyl)phenyl)benzamide (**3b**)*

Brown solid; reaction time 30 min (0.590 g, 94%); mp > 300 °C; IR (KBr): 837, 1107, 1147, 1307, 1398, 1516, 1591, 3307 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.49 (s, 3H), 6.80 (d, 2H, *J* = 8.0 Hz), 7.34–8.20 (m, 18H), 9.08 (s, 1H), 10.59 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.8, 115.1, 120.1, 123.3, 124.9, 125.3, 125.5, 125.7, 126.3, 127.6, 127.8, 128.2, 128.2, 128.3, 128.6, 129.6, 130.2, 130.2, 130.6, 131.6, 132.0, 132.5, 132.6, 133.3, 134.0, 134.2, 136.2, 136.4, 137.7, 137.8, 143.3, 144.6, 147.1, 166.1, 167.3, 175.9; HRMS (EI) *m/z:* Calcd for $C_{36}H_{25}N_3O_4S_2$: 627.7314 found: 627.7311.

*4-methyl-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)-phenylsulfonyl)phenyl)benzamide (**3c**)*

Brown solid; reaction time 30 min (0.580 g, 93%); mp > 300 °C; IR (KBr): 831, 1107, 1149, 1251, 1305, 1404, 1502, 1591, 1652, 1670, 3417 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.50 (s, 3H), 6.80 (d, 2H, *J* = 8.3 Hz), 7.27 (d, 2H, *J* = 8.0 Hz), 7.32 (d, 2H, *J* = 8.3 Hz), 7.47 (t, 1H, *J* = 6.8 Hz), 7.51 (t, 1H, *J* = 6.8 Hz), 7.66 (d, 2H, *J* = 8.5 Hz), 7.74 (d, 1H, *J* = 6.8 Hz), 7.76 (d, 1H, *J* = 7.0 Hz), 7.88 (d, 1H, *J* = 6.8 Hz), 7.93 (t, 1H, *J* = 6.5 Hz), 7.98 (d, 2H, *J* = 9.0 Hz), 8.19 (d, 1H, *J* = 7.3 Hz), 8.49 (t, 1H, *J* = 6.0 Hz), 8.81 (s, 1H), 8.87 (d, 2H, *J* = 8.5 Hz), 10.52 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.9, 125.3, 125.5, 125.7, 126.8, 127.6, 127.8, 127.9, 128.2, 128.6, 128.9, 128.9, 128.9, 129.0, 129.0, 129.2, 129.3, 130.1, 130.2, 130.6, 131.4, 131.5, 131.6, 132.0, 132.6, 134.0, 136.3, 137.8, 142.0, 142.7, 142.9, 143.3, 144.5, 145.0, 165.7, 178.0; HRMS (EI) *m/z:* Calcd for $C_{36}H_{25}N_3O_4S_2$: 627.7314 found: 627.7312.

*3-nitro-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)-phenylsulfonyl)phenyl)benzamide (**3d**)*

Brown solid; reaction time 30 min (0.610 g, 93%); mp > 300 °C; IR (KBr): 1143, 1301, 1521, 1593, 1650, 3390 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 6.81 (d, 2H, *J* = 8.5 Hz), 7.48 (t, 1H, *J* = 6.3 Hz), 7.56 (t, 1H, *J* = 6.4 Hz), 7.67 (d, 2H, *J* = 8.3 Hz), 7.69 (d, 1H, *J* = 6.0 Hz), 7.75 (t, 1H, *J* = 5.9 Hz), 7.83 (t, 1H, *J* = 6.3 Hz),7.87 (d, 2H, *J* = 8.0 Hz), 7.99 (d, 2H, *J* = 8.0 Hz) 8.19 (d, 1H, *J* = 6.3 Hz), 8.36 (d, 1H, *J* = 7.2 Hz), 8.41 (d, 1H, *J* = 7.0 Hz), 8.45 (t, 1H, *J* = 5.0 Hz), 8.77 (t, 1H, *J* = 6.2 Hz), 8.82 (s, 1H), 8.90 (d, 2H, *J* = 8.0 Hz), 10.93 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 115.1, 120.4, 122.5, 123.3, 123.6, 125.3, 125.5, 125.7, 126.4, 127.2, 127.5, 127.9, 128.2, 128.7, 130.1, 130.2, 130.5, 130.7, 131.5, 132.0, 132.6, 134.0, 134.3, 135.3, 135.6, 137.0, 137.8, 142.7, 144.5, 147.2, 147.7, 163.8, 175.9; HRMS (EI) *m/z:* Calcd for $C_{35}H_{22}N_4O_6S_2$: 658.7023 found: 658.5412.

*4-nitro-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino) phenylsulfonyl)phenyl)benzamide (**3e**)*

Brown solid; reaction time 30 min (0.615 g, 94%); mp > 300 °C; IR (KBr): 1109, 1153, 1307, 1525, 1593, 1650, 1670, 3284 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.81 (d, 2H, J = 8.8 Hz), 7.49 (t, 1H, J = 6.3 Hz), 7.52 (t, 1H, J = 6.5 Hz), 7.67 (d, 2H, J = 8.5 Hz), 7.76 (d, 1H, J = 6.8 Hz), 7.83 (d, 1H, J = 6.0 Hz), 7.87 (t, 1H, J = 6.8 Hz),7.94 (d, 1H, J = 6.0 Hz), 7.98 (d, 2H, J = 8.5 Hz), 8.15–8.21 (m, 4H), 8.30 (t, 1H, J = 6.2 Hz), 8.37 (d,2H, J = 8.0 Hz), 8.82 (s, 1H), 8.88 (d, 1H, J = 7.3 Hz), 10.91 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 115.1, 120.3, 123.3, 123.5, 123.6, 125.3, 125.5, 125.7, 126.3, 127.5, 127.9, 128.2, 128.7, 129.3, 130.0, 130.2, 130.6, 130.7, 131.5, 132.0, 132.7, 134.0, 137.1, 137.8, 139.9, 142.7, 144.0, 144.6, 147.2, 149.3, 164.4, 175.9; HRMS (EI) *m/z:* Calcd for $C_{35}H_{22}N_4O_6S_2$: 658.7023 found: 658.7011.

*3,4-dimethoxy-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino) phenylsulfonyl)phenyl)benzamide (**3f**)*

Brown solid; reaction time 30 min (0.630 g, 94%); mp > 300 °C; IR (KBr): 1149, 1255, 1309, 1498, 1589, 3292 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 3.83 (s, 6H), 6.78 (d, 2H, J = 8.0 Hz), 7.02 (d, 2H, J = 8.5 Hz), 7.42 (d, 1H, J = 6.8 Hz), 7.48 (t, 1H, J = 6.0 Hz), 7.54 (t, 1H, J = 6.3 Hz), 7.60 (d, 1H, J = 7.5 Hz), 7.67 (d, 2H, J = 8.0 Hz), 7.75 (d, 1H, J = 6.0 Hz), 7.87 (d, 2H, J = 8.0 Hz), 7.90 (t, 1H, J = 6.8 Hz), 7.92 (d, 2H, J = 8.0 Hz), 7.96 (t, 1H, J = 8.0 Hz), 8.01 (d, 1H, J = 6.3 Hz), 8.81 (s, 1H), 8.88 (d, 1H, J = 7.8 Hz), 10.39 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 30.6, 55.4, 110.9, 110.9, 111.1, 111.8, 115.1, 120.0, 121.3, 123.1, 123.3, 123.3, 125.5, 125.7, 126.2, 127.6, 127.8, 128.2, 128.6, 130.2, 130.3, 130.6, 131.5, 132.0, 132.6, 134.0, 136.2, 137.8, 143.4, 144.6, 147.1, 148.3, 151.9, 165.3, 176.0; HRMS (EI) *m/z:* Calcd for $C_{37}H_{27}N_3O_6S_2$: 673.7567 found: 673.7566.

*N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl) phenyl)acetamide (**3g**)*

Brown solid; reaction time 30 min (0.510 g, 93%); mp > 300 °C; IR (KBr): 835, 1103, 1143, 1300, 1404, 1498, 1537, 1591, 1652, 1683, 3263 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.05 (s, 3H), 6.79 (d, 2H, J = 9.0 Hz), 7.48 (t, 1H, J = 6.3 Hz), 7.56 (t, 1H, J = 6.5 Hz), 7.63 (d, 2H, J = 8.8 Hz), 7.74 (d, 2H, J = 8.8 Hz), 7.78 (d, 2H, J = 8.8 Hz), 7.83 (t, 1H, J = 6.0 Hz), 7.90 (t, 1H, J = 6.0 Hz), 7.98 (d, 1H, J = 6.0 Hz), 8.19 (d, 1H, J = 7.5 Hz), 8.80 (s, 1H), 8.85 (d, 1H, J = 6.8 Hz), 8.89 (d, 1H, J = 6.8 Hz), 10.37 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.0, 115.1, 118.8, 122.0, 123.3, 125.3, 125.5, 125.7, 127.5, 128.0, 128.2, 128.6, 130.1, 130.3, 130.6, 131.5, 132.0, 132.6, 133.5, 134.0, 135.8, 137.8, 143.2, 144.5, 147.0, 168.9, 175.9; HRMS (EI) *m/z:* Calcd for $C_{30}H_{21}N_3O_4S_2$: 551.6354 found: 551.6354.

*2-chloro-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino) phenylsulfonyl)phenyl)acetamide (**3h**)*

Brown solid; reaction time 30 min (0.550 g, 94%); mp > 300 °C; IR (KBr): 889, 1105, 1145, 1249, 1300, 1462, 1500, 1533, 1591, 1616, 1693, 3269 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 4.28 (s, 2H), 6.80 (d, 2H, J = 8.9 Hz), 7.49 (t, 1H, J = 6.0 Hz), 7.58 (t, 1H, J = 6.2 Hz), 7.64 (d, 2H, J = 8.0 Hz), 7.81 (d, 2H, J = 8.5 Hz), 7.83 (t, 1H, J = 8.8 Hz), 7.87 (t, 1H, J = 6.8 Hz), 8.19 (d, 1H, J = 6.0 Hz), 8.21 (d, 1H, J = 6.0 Hz), 8.40 (d, 1H, J = 5.8 Hz), 8.82 (s, 1H), 8.83 (d, 2H, J = 6.2 Hz), 8.88 (d, 1H, J = 8.5 Hz), 10.75 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 43.4, 115.1, 119.3, 123.3, 125.3, 125.5, 125.7, 127.5, 128.1, 128.2, 128.6, 130.0, 130.2, 130.7, 131.5, 132.0, 132.6, 134.0, 136.7, 137.8, 142.4, 144.5, 147.2, 165.2, 175.9; HRMS (EI) *m/z:* Calcd for $C_{30}H_{20}ClN_3O_4S_2$: 586.0805 found: 586.0803.

*N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl) phenyl)-4-(trifluoromethoxy)benzamide (**3i**)*

Brown solid; reaction time 30 min (0.650 g, 94%); mp > 300 °C; IR (KBr): 1149, 1255, 1502, 1526, 1591, 1671 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.81 (d, 2H, J = 8.0 Hz), 7.45–8.08 (m, 16H), 8.21 (d, 1H, J = 6.8 Hz), 8.81 (s, 1H), 8.87 (d, 1H, J = 6.8 Hz), 10.69 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 28.9, 115.1, 120.1, 120.6, 121.1, 123.3, 125.3, 125.5, 125.7, 127.5, 127.9, 128.2, 128.6, 130.2, 130.6, 131.6, 132.0, 132.6, 133.4, 133.9, 134.4, 136.7, 137.8, 143.0, 144.5, 147.1, 150.6, 164.7, 175.9; HRMS (EI) *m/z:* Calcd for $C_{36}H_{22}F_3N_3O_5S_2$: 697.7021 found: 697.7012.

*N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl) phenyl)-4-pentylbenzamide (**3j**)*

Brown solid; reaction time 30 min (0.640 g, 94%); mp > 300 °C; IR (KBr): 893, 1107, 1151, 1251, 1307, 1400, 1500, 1591, 1622, 1660, 2924, 3271 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 0.83 (t, 2H, J = 6.8 Hz), 1.22 (t, 2H, J = 7.0 Hz), 1.55 (t, 2H, J = 7.2 Hz), 2.62 (t, 2H, J = 7.2 Hz), 3.36 (s, 3H), 6.77 (d, 2H, J = 8.0 Hz), 7.33 (d, 2H, J = 8.0 Hz), 7.48 (t, 1H, J = 8.0 Hz), 7.56 (t, 1H, J = 7.8 Hz), 7.66 (d, 2H, J = 8.0 Hz), 7.76 (d, 1H, J = 6.8 Hz), 7.86 (d, 7H, J = 8.5 Hz), 7.90 (t, 1H, J = 6.8 Hz), 7.97 (t, 1H, J = 6.9 Hz), 8.19 (d, 1H, J = 8.0 Hz), 8.81 (s, 1H), 8.88 (d, 1H, J = 6.3 Hz), 10.50 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.8, 21.8, 30.2, 30.7, 34.8, 115.1, 120.0, 123.3, 125.3, 125.5, 125.7, 127.8, 127.8, 128.2, 128.6, 129.2, 130.1, 130.2, 131.5, 131.7, 132.0, 132.6, 134.0, 136.3, 137.8, 143.3, 146.8, 147.1, 165.8, 178.0; HRMS (EI) *m/z:* Calcd for $C_{40}H_{33}N_3O_4S_2$: 683.8377 found: 683.8372.

*4-methyl-3-nitro-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino) phenylsulfonyl)phenyl)benzamide (**3k**)*

Brown solid; reaction time 30 min (0.631 g, 94%); mp > 300 °C; IR (KBr): 839, 1107, 1147, 1249, 1301, 1404, 1525, 1591, 1652, 1670, 3417 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.07 (s, 3H), 6.77 (d, 2H, J = 8.2 Hz), 7.43–8.40 (m, 16H), 8.55 (s, 1H), 8.81 (s, 1H), 10.77 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 19.5, 115.1, 120.3, 123.3, 123.6, 124.9, 125.3, 125.5, 125.7, 127.9, 128.2, 128.7, 130.1, 130.2, 131.5, 132.0, 132.2, 132.7, 133.1, 133.3, 134.0, 136.6, 136.9, 142.8, 147.2, 163.7, 170.1; HRMS (EI) *m/z:* Calcd for $C_{36}H_{24}N_4O_6S_2$: 672.7589 found: 672.7284.

*4-ethyl-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino) phenylsulfonyl)phenyl)benzamide (**3l**)*

Brown solid; reaction time 30 min (0.601 g, 94%); mp > 300 °C; IR (KBr): 833, 1105, 1147, 1251, 1303, 1502, 1591, 1653, 1670, 2360, 3448 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 1.16 (t, 3H, J = 8.0 Hz), 2.62 (m, 2H), 6.80 (d, 2H, J = 9.0 Hz), 7.35 (d, 2H, J = 8.0 Hz), 7.48 (t, 1H, J = 6.0 Hz), 7.55 (t, 1H, J = 6.0 Hz), 7.66 (d, 2H, J = 8.0 Hz), 7.77 (d, 1H, J = 6.0 Hz), 7.80 (d, 2H, J = 8.0 Hz), 7.83 (d, 2H, J = 8.8 Hz), 7.84 (t, 1H, J = 6.2 Hz), 7.86 (d, 2H, J = 8.3 Hz), 8.19 (d, 1H, J = 8.1 Hz), 8.44 (t, 1H, J = 6.1 Hz), 8.81 (s, 1H), 8.85 (d, 1H, J = 6.1 Hz), 8.87 (d, 1H, J = 8.2 Hz),10.52 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 28.0, 30.6, 115.1, 120.0, 123.3, 125.3, 125.5, 125.7, 126.5, 127.5, 127.6, 127.7, 127.8, 127.9, 128.2, 128.6, 129.3, 130.1, 130.2, 130.6, 131.5, 131.6, 131.7, 132.0, 132.6, 133.5, 134.0, 136.3, 137.8, 143.3, 144.3, 147.1, 148.1, 165.8, 175.9; HRMS (EI) *m/z:* Calcd for $C_{37}H_{27}N_3O_4S_2$: 641.7579 found: 641.7579.

*4-heptyl-N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino) phenylsulfonyl)phenyl)benzamide (**3m**)*

Brown solid; reaction time 30 min (0.662 g, 93%); mp > 300 °C; IR (KBr): 891, 1105, 1149, 1303, 1498, 1591, 1668, 2850, 2922, 3278 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 0.82–1.56 (m, 12H), 2.61 (t, 3H, J = 6.8 Hz), 6.80 (d, 2H, J = 8.2 Hz), 7.32 (d, 2H, J = 8.5 Hz), 7.48 (t, 1H, J = 6.0 Hz), 7.56 (t, 1H, J = 6.2 Hz), 7.66 (d,

2H, J = 9.0 Hz), 7.75 (t, 1H, J = 5.8 Hz), 7.82 (d, 3H, J = 8.3 Hz), 7.84 (d, 3H, J = 8.5 Hz), 7.90 (t, 1H, J = 6.8 Hz), 7.95 (d, 2H, J = 8.7 Hz), 8.19 (d, 1H, J = 7.3 Hz), 8.81 (s, 1H), 8.88 (d, 1H, J = 7.4 Hz), 10.50 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.8, 21.9, 28.4, 28.9, 30.6, 31.1, 34.9, 115.1, 120.0, 123.3, 125.3, 125.5, 125.7, 127.1, 127.5, 127.8, 127.8, 128.2, 128.3, 128.6, 130.1, 130.2, 131.5, 131.6, 131.7, 132.0, 132.6, 134.0, 136.3, 137.8, 143.3, 144.5, 146.8, 147.1, 165.8, 175.9; HRMS (EI) *m/z:* Calcd for $C_{42}H_{37}N_3O_4S_2$: 711.8908 found: 711.8901.

*N-(4-(4-(5-oxo-5H-benzo[a]phenothiazin-6-ylamino)phenylsulfonyl) phenyl)-3,5-bis(trifluoromethyl)benzamide (**3n**)*

Brown solid; reaction time 30 min (0.702 g, 94%); mp > 300 °C; IR (KBr): 1139, 1280, 1598, 3271 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 6.81 (d, 2H, J = 8.2 Hz), 7.49 (t, 1H, J = 6.3 Hz), 7.56 (t, 1H, J = 6.5 Hz), 7.67 (d, 2H, J = 8.8 Hz), 7.76 (d, 1H, J = 6.8 Hz), 7.81 (t, 1H, J = 5.5 Hz), 7.90 (d, 2H, J = 8.8 Hz), 7.93 (d, 2H, J = 8.9 Hz), 7.99 (t, 1H, J = 6.1 Hz), 8.19 (d, 1H, J = 6.0 Hz), 8.30 (s, 1H), 8.42 (d, 1H, J = 6.2 Hz), 8.58 (s, 2H), 8.82 (s, 1H), 8.88 (d, 1H, J = 6.3 Hz), 10.95 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 28.1, 30.6, 115.1, 120.5, 123.3, 125.3, 125.5, 125.7, 127.5, 127.9, 128.2, 128.7, 129.5, 130.0, 130.2, 130.6, 131.5, 132.0, 132.7, 134.0, 136.6, 137.2, 137.8, 142.5, 144.6, 147.2, 163.0, 175.9; HRMS (EI) *m/z:* Calcd for $C_{37}H_{21}F_6N_3O_4S_2$: 749.7007 found: 749.7006.

Molecular docking studies

To understand the interaction of all the synthesized molecules (**1**, **2**, **3a–n**) with HDAC8 and *Bacillus subtilis*, the crystal structure of HDAC8 (Histone deacetylase 8) with SAHA (Suberoylanilide Hydroxamic Acid) [19] and crystal structure of YmaH from *B. subtilis* [20] were downloaded from protein data bank and the molecular docking studies were performed using the GLIDE program [21] (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 the protocol was evaluated by re-docking. SAHA (PDB ID: 1T69) and YmaH (PDB ID: 3HSB) were selected for docking studies as a reference sample and was prepared for docking through a protein preparation wizard. Structures of **1**, **2**, **3a–n** were sketched using ACD/chemsketch (Freeware version). A GLIDE grid generation wizards have been used to define the docking space. Docking was performed using XP (Extra Precision mode) docking protocol.

Measurement of cytotoxicity

To evaluate the cytotoxic property of the synthesized quinone derivatives, the MTT assay was carried out [22]. A stock solution of 20 mg/mL was prepared in dimethyl sulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO, USA). The solution was stored in aliquots at −20 °C. Further dilutions were made in Dulbecco's Modified Eagle Medium (DMEM) to required concentrations of 5–150 μg for the treatment of SiHa cells. The samples were dissolved in DMSO. The human cervical cancer cells were seeded in 96-well plates at a density of 1×10^4 cells/well and treated with the synthesized quinone derivatives at different concentrations. After incubation, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline (PBS) were added to each well. The plates were wrapped with aluminum foil and incubated for 4 h at 37 °C. The plates were centrifuged and purple formazan product was dissolved by the addition of 100 μL of DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, CA, USA). Data was collected for three replicate each and the mean was calculated. The percentage inhibition was calculated, from the data, using the formula given below, and IC_{50} values were calculated using nonlinear regression analysis.

$$\frac{\text{Mean OD of untreated cells(control)-Mean OD of treated cells} \times 100}{\text{Mean OD of untreated cells(control)}}$$

The IC_{50} concentration was determined as the dose that needs to be required to kill 50% of the cells.

In vitro antibacterial activity

All the synthesized compounds were studied for their antibacterial activity against clinically isolated two Gram-positive bacteria (*B. subtilis* and *Staphylococcus aureus*) and five Gram-negative bacilli (*Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeruginosa*) using conventional agar-dilution method [23,24]. The minimum inhibitory concentrations (MICs) values were calculated by comparison between Sparfloxacin and Norfloxacin as the standard antibacterial drugs and they are presented in Table 3. 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 a 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 1.5×10^5 CFU/mL. The synthesized compounds and standard antibacterial drugs (50 mg) were dissolved in dimethyl formamide (DMF) (0.5 mL) and the solution was diluted with water (4.5 mL) to get a stock solution of 10000 mg/L for each compound. Further progressive double dilution with Muller Hinton broth was performed to obtain the required concentrations of 2500–0.4 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 °C was done and the growth measured. The MICs of the test compounds and the standard control drugs are tabulated in Table 3.

Results and discussion

Chemistry

2,3-Dichloro-1,4-naphthoquinone reacts with 4-amino phenyl sulfone to produce 6-(4-(4-aminophenylsulfonyl)phenylamino)-3-chloronaphthalene-1,4 dione (**1**). Previously compound **1** preparation was reported by Carroll et al. [18] following a difficult procedure. According to them 2,3-dichloro-1,4-naphthoquinone react with hydrochloride salt of 4-amino phenyl sulfonamide in absolute ethanol media and in the presence of N,N-diethyl aniline acting as the catalyst and the reaction was performed for 18 h under reflux condition. The overall percentage yield of compound **1** was reported to be only 72%. In our previous report we synthesized compound **1** by a simple and green route to achieve a yield of 88% [17]. In this method, 2,3-dichloro-1,4-naphthoquinone was made to react with 4-amino phenyl sulfone (no hydrochloride salts of sulfonamides) in water as solvent and refluxing the mixture for 2 h. After cooling the reaction mixture at room temperature the precipitate was filtered by *vacuum* filtration and crystallized in 95% absolute ethanol. In comparison with the method of Carroll et al., our method had the advantage of giving better yields and also the preparative steps were green. Compound **2** were synthesized from compound **1**, when compound **1** were reacted with an equal amount of 2-amino thiophenol in absolute ethanol media and the mixture refluxed for 2 h. We attempted

Table 1
Molecular docking data of compounds **1**, **2**, **3a–h** with HDAC8 protein receptor.

Compounds	Molecular docking				IC_{50} of SiHa (μg/mL)
	Glide score (kcal/mol)	E model score	Glide energy (kcal/mol)	XP H Bond (Å)	
1	−4.20	−48.68	−38.56	−0.51	85
2	−3.26	−55.82	−38.90	−0.73	105
3a	**−8.08**	**−78.05**	**−65.95**	**−0.88**	**26**
3b	−9.43	−69.90	−68.73	−1.00	65
3c	−7.87	−57.14	−64.77	−0.35	115
3d	−8.68	−86.44	−72.29	−0.90	73
3e	−8.69	−69.94	−62.29	−1.01	100
3f	−9.24	−73.42	−75.66	−1.46	107
3g	−5.76	−90.41	−74.10	−0.86	108
3h	−7.59	−98.61	−75.34	−0.87	110
3i	−9.03	−52.14	−64.75	−0.00	44
3j	−8.92	−59.02	−66.53	−0.19	57
3k	−8.27	−67.27	−63.20	−0.00	96
3l	−5.09	−65.21	−50.35	−0.70	53
3m	−4.82	−68.84	−52.63	−0.00	82
3n	**−4.78**	**−64.50**	**−44.03**	**−0.00**	**32**
SAHA	−9.42	−84.04	−63.82	−1.43	–

Bold letters indicates better activity.
– Cytotoxicity not studied

Table 2
Molecular docking studies of sixteen analogs taken for study with *Bacillus subtilis* (PDB ID: 3HSB).

Compounds	Molecular docking		
	Glide score (kcal/mol)	E model score	MIC of BS (μg/mL)
1	**−4.656**	**−64.77**	**1.4**
2	−4.597	−73.05	625
3a	−3.903	−81.85	316
3b	−2.879	−88.42	312.5
3c	−4.580	−60.17	315
3d	−4.817	−92.26	*
3e	−4.708	−95.10	156.25
3f	−4.208	−83.85	78
3g	−4.745	−79.54	6.9
3h	−5.433	−77.89	279
3i	−5.277	−94.51	78
3j	−5.680	−97.16	156.25
3k	−5.743	−90.31	94
3l	**−4.169**	**−92.14**	**0.6**
3m	2.714	−87.10	5
3n	**−6.143**	**−90.91**	**0.4**
Sparfloxacin[a]	–	–	9.76
Norfloxacin[a]	–	–	*

– Docking studies not carried out.
* No inhibition observed.
Bold letters indicates better activity.
[a] Standard antibacterial drugs.

the same reaction in water media but we were not successful in the formation of a number of inseparable compounds. The compounds **3a–n** was synthesized from compound **2**, when it was made to react with substituted acid chlorides in acetone media without the aid of any base. In a previous report [26], the aminophenylsulfone is dissolved in pyridine and the substituted acid chlorides are dissolved in dioxane and added drop wise to the reaction mixture and the reaction mixture was stirred overnight at room temperature. However in our reaction the compound **2** reacted with the substituted acid chlorides in the acetone medium under reflux condition for 30 min and the overall percentage yield was between 95–98%. (See Scheme 1)

This is the first report on the synthesis of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives (**3a–n**) to the best of our knowledge. Very few reports are available in the literature about compounds with phenothiazin moiety [27]. The molecular docking, antibacterial studies against different Gram-positive and Gram-negative pathogens and cytotoxicity effect against cervical cancer cell line (SiHa) studies have not been attempted on these systems so far.

Molecular docking studies of quinone derivatives

Aromatic carbonyl functional groups of all the molecules (**3a–n**) was found to be close to Zn^{2+} atom in the active site, and established the hydrogen bond with GLY 151 which is the major interactions of the ligands with HDAC8 (see Table 1). To understand the interaction of bacterial protein receptor with synthesized molecules (**1**, **2**, **3a–n**) the crystal structure of YmaH from *B. subtilis* was downloaded from protein data bank and studied with the glide program. All the glide and the E model score is compared with the MIC of *B. subtilis*, for the tested compounds and 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* cytotoxicity and antibacterial study results show that the glide scores, IC_{50} 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 inactive) [21,28] (see Figs. 1–4).

In vitro cytotoxicity properties of quinone derivatives

The *in vitro* cytotoxic activity of the selected synthesized compounds (**1**, **2** and **3a–n**) was evaluated by a cell viability assay method against a human cervical cancer cell line (SiHa). All the cytotoxicity values are reported as IC_{50} (μg/mL) and presented in Fig. 5 and these values were compared with the standard drug Doxorubicin (μM). All the synthesized compounds exhibited less cytotoxic activity than Doxorubicin (2.445 μg/mL). Most of the derivatives tested to show enhanced cancer cell growth except the compounds **3a** (26 μg/mL), **3n** (32 μg/mL), **3i** (44 μg/mL), **3l** (53 μg/mL), **3b** (65 μg/mL), **3d** (**73** μg/mL) and **1** (85 μg/mL). Among all the molecules studied the compound **3a** (26 μg/mL) and **3n** (32 μg/mL) exhibited better cytotoxic activity. In our previous report [31] the same kind of moiety reported for their cytotoxic property. Compounds posses electronegative atoms at ZBR exhibits better cytotoxicity. In this report compound **3n** posses two trifluoro methyl ($-CF_3$) functional groups and exhibit remarkable cytotoxic activity.

Table 3
In vitro antibacterial activity of phenothizin-5-one derivatives against Gram-positive and Gram-negative bacteria (MICs in μg/mL).

Compounds	MIC (μg/mL)						
	B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*	*S. typhi*	*P. aeruginosa*	*K. pneumoniae*
1	1.4	**34**	762	*	*	99.2	1015
2	625	625	312.5	78	**76**	156.25	**312.5**
3a	316	625	235.3	175.2	392.3	172.3	578
3b	312.5	625	312.5	312.5	156.25	156.25	625
3c	315	618	214.7	162	382.1	168.1	563
3d	*	625	156.25	312.5	276.5	376.2	318.4
3e	156.25	156.25	156.25	312.5	*	78	463.15
3f	78	79	156.25	*	*	156.25	154.25
3g	6.9	523	345	378	412	405	612
3h	279	731	275	212	413	387	573
3i	78	156.25	*	156.25	312.5	312.5	314.5
3j	156.25	143.5	**39**	42	78	*	463.15
3k	94	576.3	424.8	173.4	327.2	163.7	578
3l	**0.6**	26.2	278	*	271	**28**	867
3m	5	523	*	25	255	93.6	765
3n	**0.4**	38	731	**19**	221	91.7	845
Sparfloxacin[a]	9.76	4.87	156.3	4.8	2500	156.3	2500
Norfloxacin[a]	*	39.06	625	*	627	39.06	<1.2

Lower MIC values indicates higher antibacterial activity.
Bold letters indicates that the best activity among all compounds studied.
* No inhibition observed.
[a] Standard antibacterial drugs.

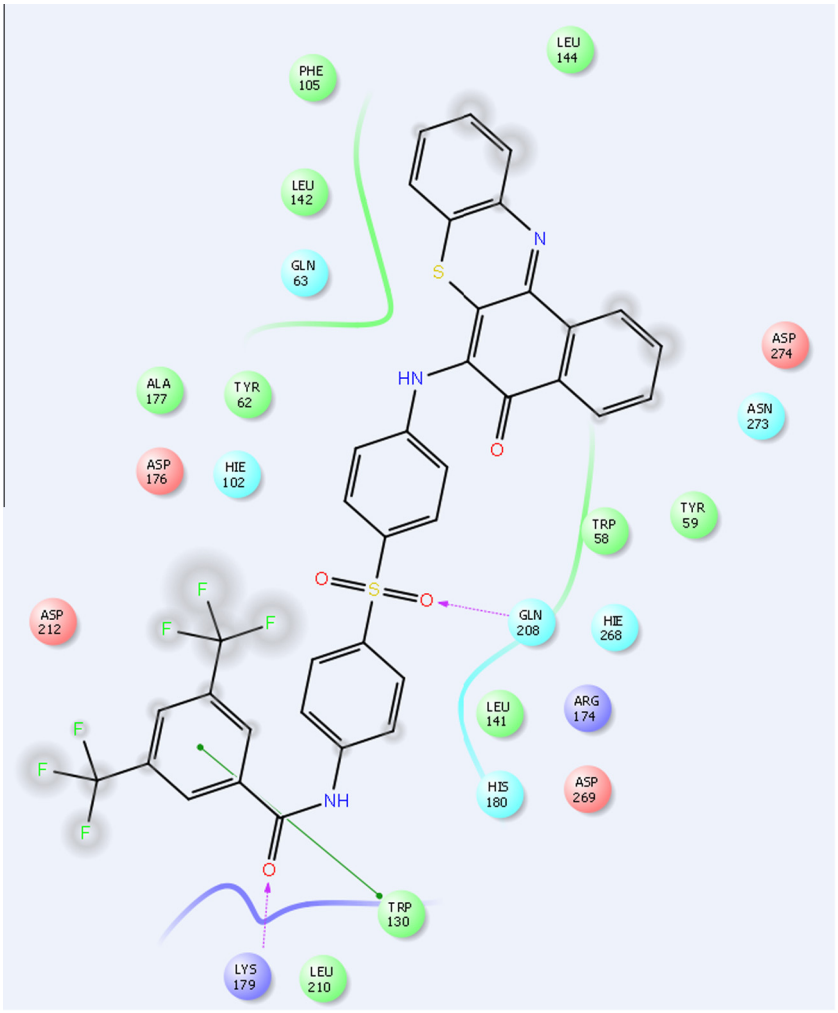


Fig. 1. Docking model structure of compound **3n** into the YmaH (PDB ID: 3HSB) binding pocket.

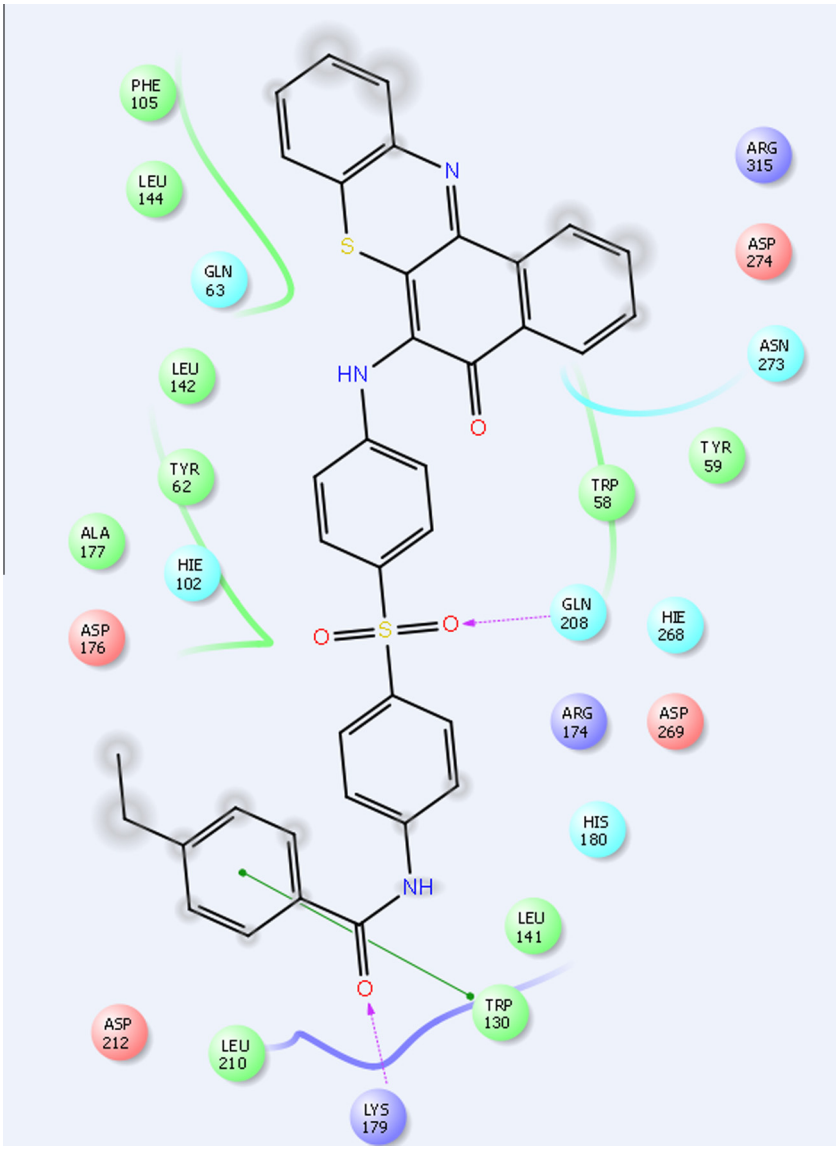


Fig. 2. Docking model structure of compound **3l** into the YmaH (PDB ID: 3HSB) binding pocket.

In vitro antibacterial activity

All the synthesized compounds were tested against two Gram-positive and five Gram-negative bacteria's. All the compounds (**1**, **2**, **3a–n**) exhibited good antibacterial activity against Gram-negative bacteria of *K. pneumoniae* than the standard drugs used (Sparfloxacin and Norfloxacin). Compound **3l** exhibits good activity against most of the Gram-positive and Gram-negative microorganisms due to the presence of the ethyl group in the fourth position of the aromatic system of the benzoyl unit. Compounds **3n**, and **3l** (0.6 μg/mL), exhibits better activity against *B. subtilis* (0.4 μg/mL, 0.6 μg/mL), *S. typhi* (221 μg/mL, 271 μg/mL), *P. aeruginosa* (91.7 μg/mL, 28 μg/mL) and *K. pneumoniae* (848 μg/mL, 867 μg/mL) than Sparfloxacin and Norfloxacin. Compound **3j** (39 μg/mL) exhibits better activity against *E. coli* than the standard drugs of Sparfloxacin (156.3 μg/mL) and Norfloxacin (625 μg/mL). Compound **2** (76 μg/mL) and **3j** (78 μg/mL) exhibits better antibacterial activity against *S. typhi* than Sparfloxacin (2500 μg/mL) and Norfloxacin (627 μg/mL). Compounds **3n** (0.4 μg/mL) and **3l** (0.6 μg/mL) exhibit good activity among all the molecules synthesized against *B. subtilis* than Sparfloxacin (9.76 μg/mL). Standard drug Norfloxacin did not exhibit any activity against *B. subtilis* and *P. vulgaris* microorganisms. Compound **1** did not exhibit any inhibition against *P. vulgaris, S. typhi.* All the MIC values are tabulated and presented in Table 3.

The compounds (**3a–n**) were synthesized by target based drug discovery. The compound **1** exhibits antibacterial activity of MIC = 1.4 μg/mL against *B. subtilis*. But the introduction of acid chlorides to the $-NH_2$ group in molecule **1** exhibit better antibacterial activity against *B. subtilis* than compound **1**. Compounds **3n** (MIC = 0.4 μg/mL), **3l** (MIC = 0.6 μg/mL) posses phenothiazin moiety and ZBR (zinc binding region) exhibits better activity against *B. subtilis* among all molecules studied. So from the observation the introduction of acid chlorides in primary amine plays an important role for enhanced biological activities. The molecules exhibit better interactions with targeted proteins should posses ZBR within the molecule. But compound **1** does not have such kind of molecular similarities and this molecule was modified and posses phenothiazin and ZBR units within the molecule. In comparison with *in vitro* antibacterial activity, compound **1** did not exhibit any remarkable activity. Molecules exhibit better activity construct with phenothiazin and ZBR (eg. **3n**, **3j**, **3l** etc.,). From the *in vitro*

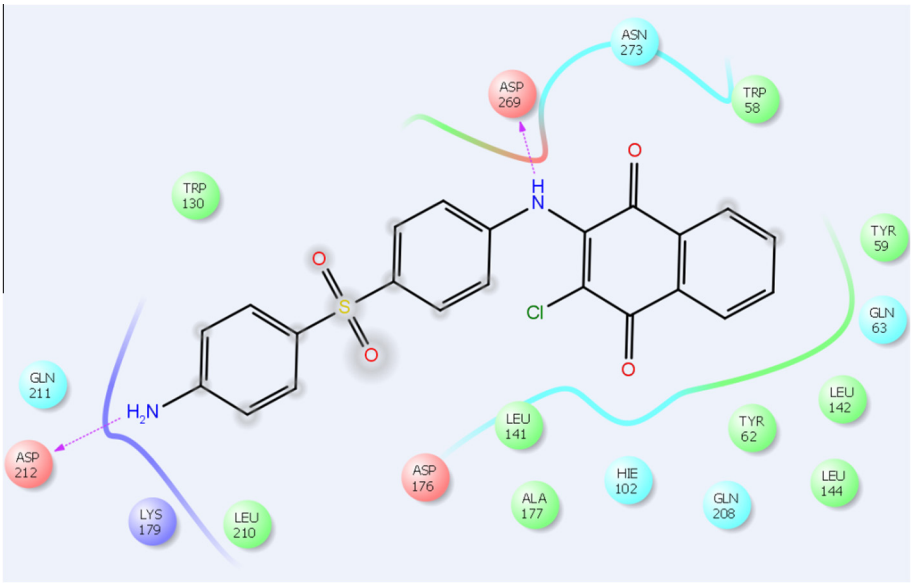


Fig. 3. Docking model structure of compounds **1** into the YmaH (PDB ID: 3HSB) binding pocket.

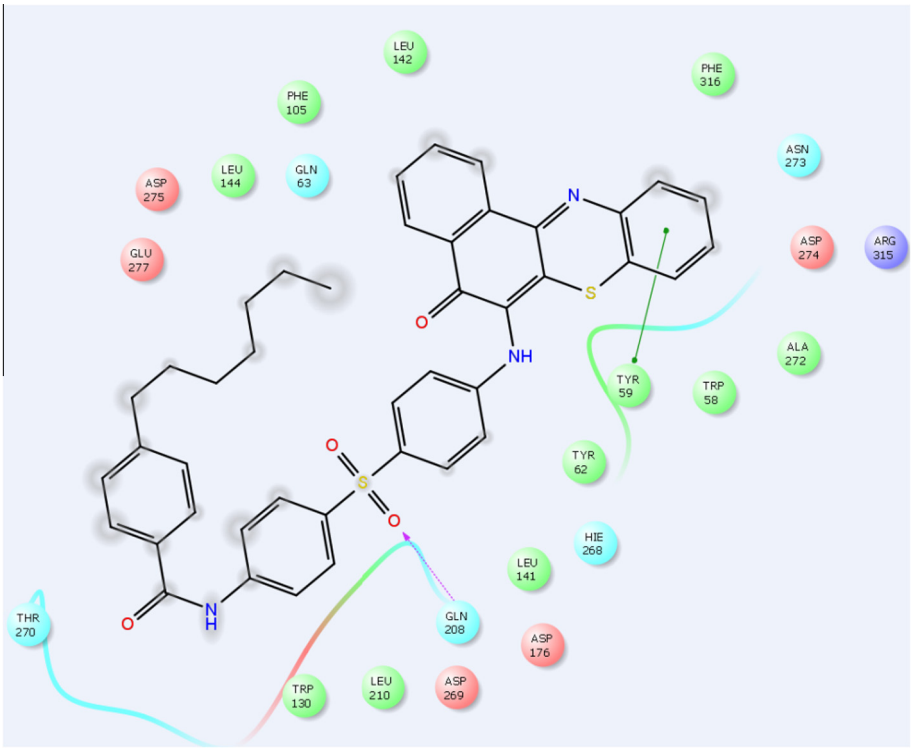


Fig. 4. Docking model structure of compound **3m** into the YmaH (PDB ID: 3HSB) binding pocket.

antibacterial and cytotoxicity studies, the phenothiazin and ZBR are most important to enhance the biological activities (see Fig. 6).

Structure activity relationship of **3a–n** revealed that compounds with aromatic halides and methyl functional groups posses better antibacterial activity. The compound **3n** (0.4 μg/mL) and **3l** (0.6 μg/mL) posses aryl methyl and aryl halides such as trifluoro methyl and ethyl ($-CF_3$, $-CH_3$, $-CH_2$) functional groups at a zinc binding region (ZBR) of the molecules exhibits better antibacterial activity. Compound **3n** have two aryl halides at 3 and 5 positions of ZBR exhibits better antibacterial activity against *B. subtilis* among all molecules studied. The compound **3l** exhibits better antibacterial activity against gram-positive and Gram-negative bacteria due to the presence of ethyl function group. The molecules even having halides at ZBR do not exhibit the remarkable antibacterial activity (**3g**, **3h**). The phenothiazin moiety also plays an important role to enhance the antibacterial activity. In summary the naphthoquinone derivatives posses with phenothazin moiety and electron donating and electronegative atoms in the aromatic ring of ZBG exhibits better antibacterial activity (see Table 3).

Conclusions

A new series of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothizin-5-one derivatives were synthesized and

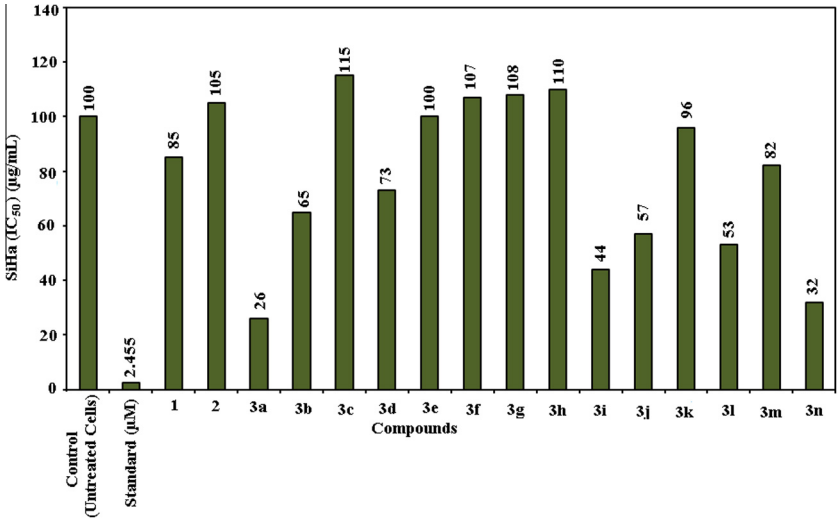


Fig. 5. Cytotoxicity (IC_{50}) of 6-(4-(4-aminophenylsulfonyl)phenylamino)-5H-benzo[a]phenothiazin-5-one derivatives (**3a–h**) and Doxorubicin (μM) used as standard.

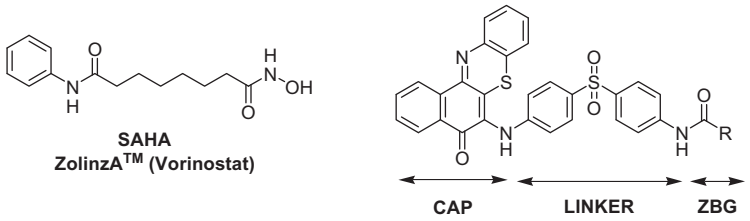


Fig. 6. Structure of SAHA and pharmacophoric features of the title compounds.

characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and high resolution mass (HRMS-EI) spectral analyses. All the molecules were studied for their interactions with target protein receptors by molecular docking protocol. In the *in vitro* cytotoxicity study of all the synthesized molecules against cervical cancer cell line (SiHa), it was found that compound **3a** (26 μg/mL) alone exhibits good IC_{50} value and most of the synthesized compounds enhance the growth of cancer cell line. In the study of *in vitro* antibacterial activity of the tested compounds it is observed that there is improved activity against all the microorganisms used. In particular compound **3n** and **3l** showed marked activity against four microorganisms. Compound **3n** (0.4 μg/mL) exhibited better activity against *B. subtilis* than even the standard drug of Sparfloxacin (9.76 μg/mL). On comparing the results of *in vitro* cytotoxicity studies and antibacterial studies it is clearly observed that, phenothiazin-5-one derivatives are very effective against Gram-positive and Gram-negative bacterial microorganism than the cancer cell line (SiHa).

Acknowledgements

The authors thank the Management and the authorities of Karunya University, Coimbatore, for their kind support, constant encouragement and providing KSJR fellowship to PR. We extend our thanks to SAIF, IIT, Madras, India and IISC, Bangalore, India for the NMR and HR-Mass spectral analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.12.036.

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