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Synthesis, molecular docking and cytotoxicity evaluation of novel 2-(4-amino-benzosulfonyl)-5H-benzo[b]carbazole-6,11-dione derivatives as histone deacetylase (HDAC8) inhibitors

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ABSTRACT

A new series of 2-(4-aminobenzosulfonyl)-5H-benzo[b]carbazole-6,11-dione derivatives, which has not been reported yet, has been synthesized from 1,4-naphthoquinone and 4-aminophenylsulfone involving an Michael addition, benzoylation and Pd catalyzed coupling. This set of compounds has been evaluated for *in vitro* cytotoxicity specifically against human cervical cancer cell line (SiHa) and most of the synthesized compounds exhibited good cytotoxic activity. Molecular docking of all the synthesized compounds was studied; among fourteen molecules docked compound **3** was the one with the best glide and E model score of −9.06 and −73.41, respectively which is close to the glide score of SAHA (standard). In all docked molecules, the compound **7a** exhibits least glide and E model score of −2.97 and −71.02 respectively.

1. Introduction

HDAC activity is invariably increased in cancer cells and there is a need to synthesize novel class of HDAC inhibitors. SAHA (Suberoyl Anilide Hydroxamic Acid, Fig. 1) is the drug has been recently validated clinically and approved by FDA for the treatment of cutaneous T cell lymphoma [1]. Methyl-Gene's isotype specific HDAC inhibitor MGCD0103 (Fig. 1) is currently clinically investigating for in solid tumors and hematological malignancies [2]. Most of HDAC inhibitors have three common features such as metal binding moiety, a carbon linker and a capping group (Fig. 1). In HDAC inhibitors, the capping group is solvent-exposed and interacts with amino acids near the entrance of the active site, metal binding moiety binds in the protein interior and complexes the metal ion involved in catalysis. The linker helps for high-affinity interactions with proteins [3].

Quinones are effectively involved in a wide range of biochemical processes including electron transport and oxidative phosphorylation [4]. Heterocyclic quinones are biologically active [5] and aminoquinones create high interest due to their enhanced anticancer activities. The heterocyclic aminoquiniones have wide range of biological applications including anticancer [6], antibacterial [7,8], fungicidic [8,9], luciferase inhibition [10], antiproliferative [11] and tuberculostatic effects [12]. In addition, the heterocyclic naphthoquinone derivatives exhibit potent properties like electrochemical capacitance [13], electrochemical redox [14], electron mediator [15] and electron transfer [16]. They are also capable of forming complexes with metals [17].

According to Moore [18] and Pindur [19], a molecule with DNA-intercalating property should have three to four coplanar rings with a length of 3–4 Å and a width of 6–8 Å. The molecule must also possess a *para*-conjugated quinone ring having nitrogen atoms, which will help it to make hydrogen bonding with DNA. The structure–activity relationship of heterocyclic quinones containing nitrogen atoms shows that the number and position of nitrogen atoms are very important for the cytotoxic activity [20]. If the number of nitrogen atoms increases, the cytotoxicity also increases [21]. The structure of carbazole-6,11-dione (**1a–7a**) targeted in the present investigation has a similar kind of ring arrangement helping it to form a hydrogen bonding with DNA and hence this investigation assumes importance.

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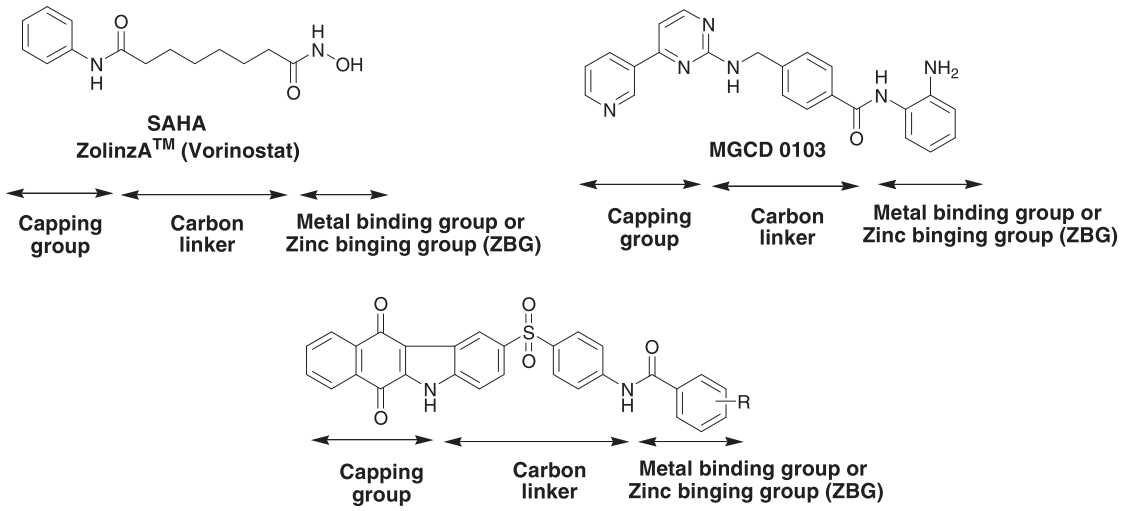


Fig. 1. Structure of SAHA, MGCD0103 and pharmacophoric features of HDAC inhibitors in the title compounds.

The second most common cancer in females worldwide has been identified to be cervical carcinoma. In a year it is calculated that 12,200 new females suffer from cervical cancer and about 4210 deaths are attributed to cervical cancer in the United States alone [25,26]. Therefore, in our effort to identify new and effective therapeutic agents for cancer treatment, selective HDAC inhibitors have to design and synthesis has become one of our major goals. In this concern we synthesized a new series of 2-(4-amino-benzosulfonyl)-5H-benzo[b]carbazole-6,11-dione derivatives and studied their cytotoxicity against human cervical cancer cell line (SiHa) and the molecular docking studies of all the synthesized compounds were studied and reported.

2. Results and discussion

2.1. Chemistry

1,4-Naphthoquinone reacts with 4-aminophenyl sulfone to generate 2-[4-(4-amino-benzenesulfonyl)-phenylamino]-[1,4]naphthoquinone (**1**). Though this conversion has already been effected in glacial acetic acid under reflux [12], it is now found that the reaction take place smoothly in water medium without the aid of the acid. It is found that the yield in this method is only marginal (41%) (Method B) [27], but when the reaction is conducted in a mixture of ethanol and acetic acid, the reaction led to a very good yield (91%) (Method A). However performing the reaction in ethanol alone is not at all successful, the yield being very poor (35%). Compound **1** is then reacted with several aromatic acid chlorides to give N-{4-[4-(1,4-dioxo-1,4-dihydro naphthalene-2-yl amino)-benzene sulfonyl]-phenyl}-aryl benzamide derivatives (**2**–**7**) by conventional method in acetone and yield between 98–99%. Finally carbazole-6,11-dione derivatives (**1a–7a**) are synthesized *via* a typical intramolecular cyclization with palladium (II) acetate in acetic acid (Method C).

This the first report on the synthesis of N-{4-[4-(1,4-dioxo-1,4-dihydronaphthalen-2-yl amino)-benzenesulfonyl]-phenyl}-aryl benzamide derivatives (**2**–**7**) and 2-(4-amino-benzosulfonyl)-5H-benzo[b]carbazole-6,11-dione derivatives (**1a–7a**) to the best of our knowledge. Only few reports are available in literature wherein compounds with carbazoloquinone nuclei on naphthoquinone and N-dansyl carbazoloquinone have been found to exhibit antituberculosis [12] apart from chemical and electrochemical fluorescent activities [22]. However, the cytotoxicity and the molecular docking have not been tested on these systems so far. The synthetic methodology of title compounds are given in the Scheme 1.

2.2. Cytotoxic properties of quinone derivatives

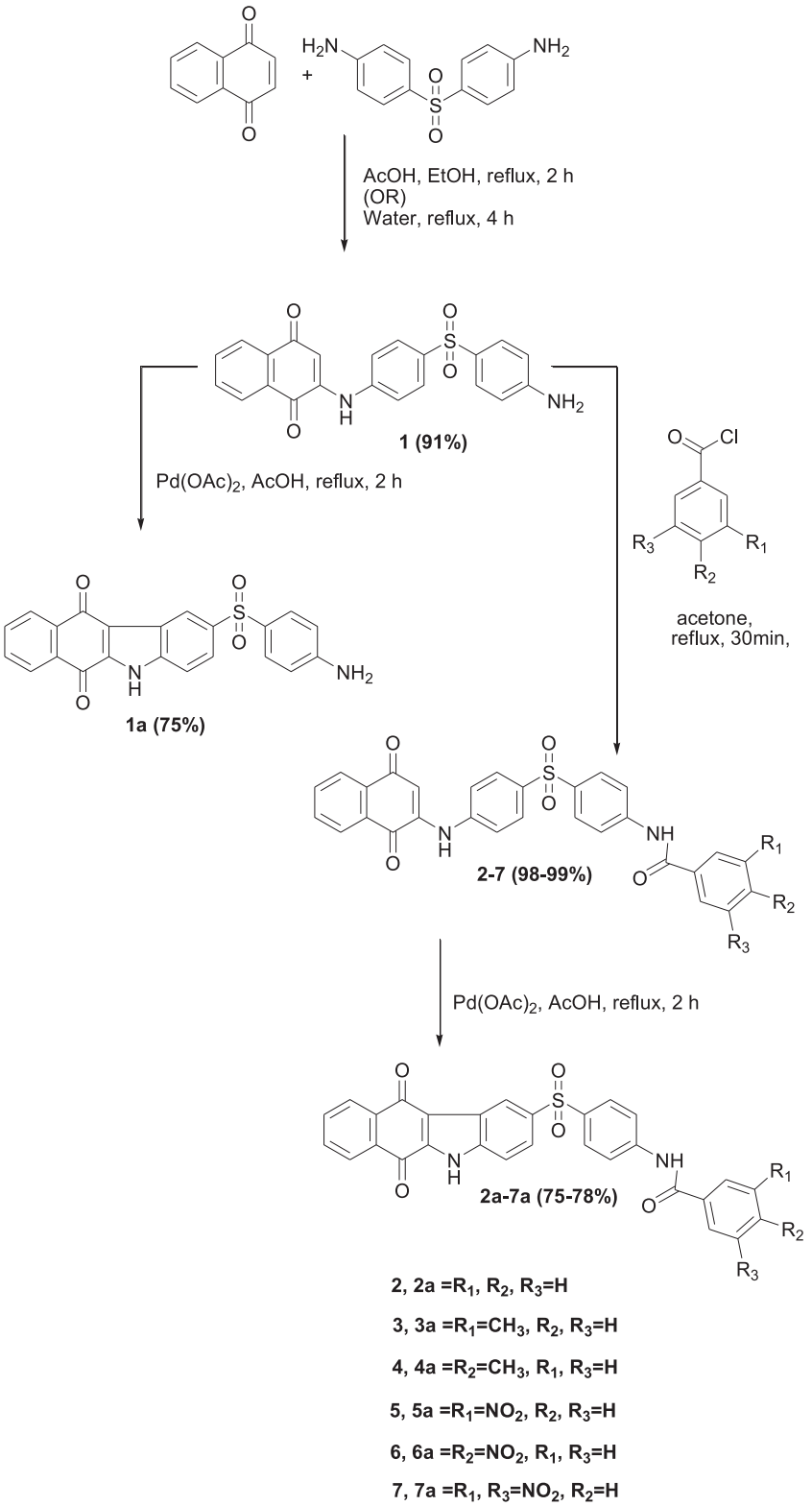
The *in vitro* cytotoxic activities of the synthesized compounds (**1**–**7**, **1a–7a**) are evaluated by cell viability assay method against a human cervical cancer cell line (SiHa). Though the synthesized compounds show potent cytotoxic activity (**2a**, **3a**, **4a**, **5a**, **7a)** than their precursors. Compounds **1a** and **6a** exhibited less cyctotoxicity than its precursors. Thus the ring closed carbazole-6,11-dione derivatives (**2a**, **3a**, **4a**, **5a**, **7a**) that consist of four coplanar annulated heterocyclic rings show higher antitumor activity than 2-[4-(4-amino-benzene sulfonyl)-phenyl amino]-[1,4] naphthoquinone derivatives (**2**, **3**, **4**, **5**, **7**).

Among **1**–**7**, it is noticed that 2-[4-(4-aminobenzenesulfonyl)-phenylamino]-[1,4] naphthoquinone (**1**) and N-{4-[4-(1,4-dioxo-1,4-dihydronaphthalene-2-ylamino)benzene sulfonyl]phenyl}-4-nitrobenzamide (**6**) have more toxicity than the other compounds with the latter having more effect than the former. All the IC_{50} values are listed in Fig. 2.

N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-3,5-dinitro-benzamide (**7a**), 2-(4-amino-benzosulfonyl)-5H-benzo[b]carbazole-6,11-dione (**1a**) and N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-4-methylbenzamide (**4a**) exhibited good cytotoxic activity in the carbazole system than the other compounds of the series (**2a**, **3a**, **5a**, **6a**). In carbazole system the compound **6a** exhibited less activity than compound **6**.

2.3. Molecular docking studies of quinone derivatives

Aromatic carbonyl functional group of all the molecules (**3a–h**) were 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 (Figs. 3–16). Among the ten molecules docked the compound **3** was the one with the best glide and E model score of −9.06 and −73.41, respectively which is close to the glide score of SAHA (standard). The compound **3** exhibited two hydrogen bonds with GLY 151 (C–O–H) and LYS 33 (N–H–O) with the bond distance of 1.855 ÅA and 2.070 ÅA respectively (Fig. 7). All the docking results are presented in Table 1. Compound **3a** comes next with a glide and E model score of −8.96 and −72.72,



Scheme 1. The synthesis of 2-[4-(4-amino-benzenesulfonyl)-phenylamino]-[1,4]naphthoquinone (**1**), N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-ylamino)-benzenesulfonyl]-phenyl}-aryl benzamides (**2**–**7**) and carbazole-6,11-dione derivatives (**1a–7a**).

respectively and it exhibited one hydrogen bond with GLY 151 with the distance of 1.825 ÅA (C–O–H) (Fig. 8). All docked molecules, the compound **7a** exhibits least glide and E model score of −2.97 and −71.02 respectively (Fig. 16).

Among fourteen molecules docked the compound **3** exhibited good glide and E model score and it reveals that the substitution at the zinc binding group plays an important role to bind with HDAC8. At the same time the absence of methyl group in carbonyl

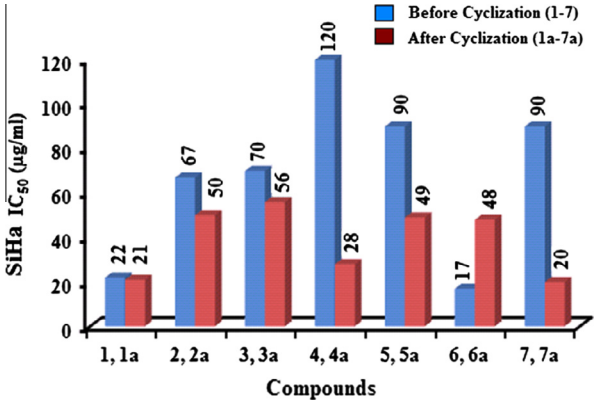


Fig. 2. Comparison of cytotoxicity (IC_{50}) of derived 2-[4-(4-amino-benzenesulfonyl)-phenyl amino]-[1,4]naphthoquinone (**1–7**) with 2-substituted 2-(4-amino-benzosulfonyl)-5H-benzo [b]carbazole-6,11-dione derivatives (**1a–7a**).

group orient the cap group differently in the pocket. These factors together has kept not favoring hydrogen bonding interaction with HDAC8 for other molecules (**1**, **1a**, **7**, **7a**), a favorable interaction shown by many HDAC Inhibitors (**3**, **3a**, **6**, **2**, **2a**). This may led to the reduced potency of **7** and **7a** compared with other docked molecules.

The use of glide and E model scores are questionable for the type of rank ordering of different derivatives within a series. The molecular docking and *in vitro* cytotoxocity study results suggested that, glide scores and IC_{50} values of the synthesized compounds did not correlated because of the glide scores mainly used to separate 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) [28,29].

3. Experimental

3.1. Materials and methods

Melting points (°C, uncorrected) of the synthesized compounds were checked in open an capillary tubes by using digital auto melting point apparatus (Labtronics 110, India) and found uncorrected. All the chemicals and solvents were purchased from Sigma–Aldrich and Merck, India. All reactions were carried out under atmospheric air and the products were checked by thin layer chromatography on TLC silica gel 60 F254 using eluting solvents such as ethyl acetate and hexane (1:1). The synthesized compounds were purified by column chromatography using column silica gel 100–200 mesh (ethyl acetate:hexane 1:2). All the compounds were characterized by UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan) using acetone as solvent, FT-IR spectrometer (IR 8400, Shimadzu, Japan) using KBr pellets, ^{1}H NMR spectroscopy in DMSO (400 MHz, Bruker), ^{13}C NMR spectroscopy in DMSO (100 MHz, Bruker) using tetramethylsilane (TMS) as internal standard. Coupling constants (*J* values) are reported in Hz. Mass spectra were measured by Electron Impact (EI) method (Jeol GC-Mate 2). *In vitro* cytotoxicity of all the compounds was studied by cell viability assay method. Molecular docking studies of all the synthesized compounds were studies by GLIDE program (version 8.5, Schrodinger, LLC, New York, 2010).

3.2. General procedures for synthesis of 2-[4-(4-amino-benzene sulfonyl)-phenyl amino]-[1,4] naphthoquinone (*1*)

3.2.1. Method A: [12]

A solution of 1,4-naphthoquinone (1.581 g, 10 mmol) in 95% of ethyl alcohol (40 mL) was gradually added over a period of 30 min, to a solution of 4-aminophenyl sulfone (2.048 g, 10 mmol) in glacial acetic acid (10–30 mL) and stirred for 30 min. Then the mixture was refluxed for 1 h. The reaction mixture was cooled and left overnight at room temperature. The black precipitate formed

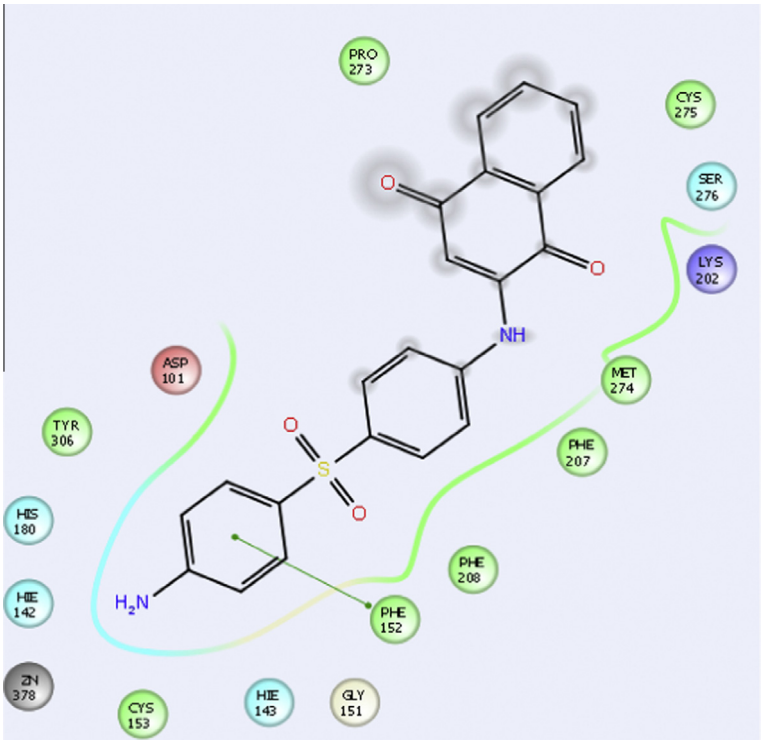


Fig. 3. Docking model structures of compound **1** into the HDAC8 binding pocket.

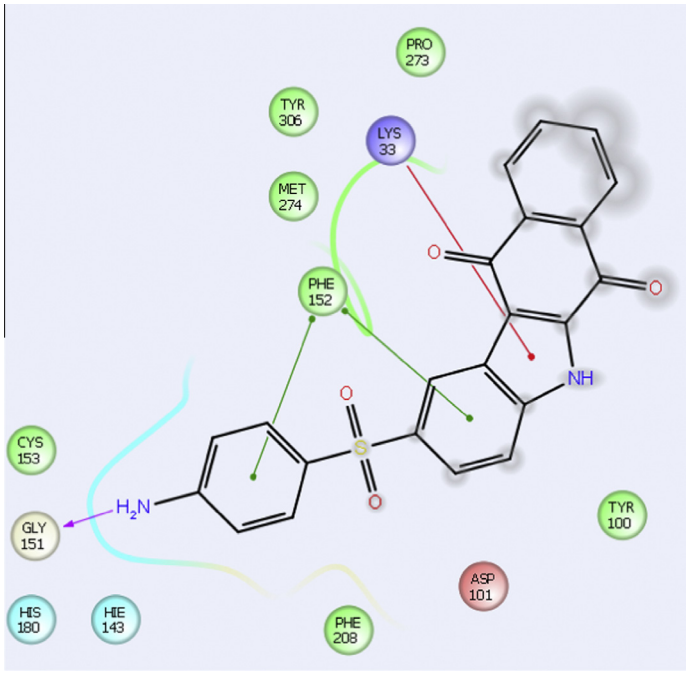


Fig. 4. Docking model structures of compound **1a** into the HDAC8 binding pocket.

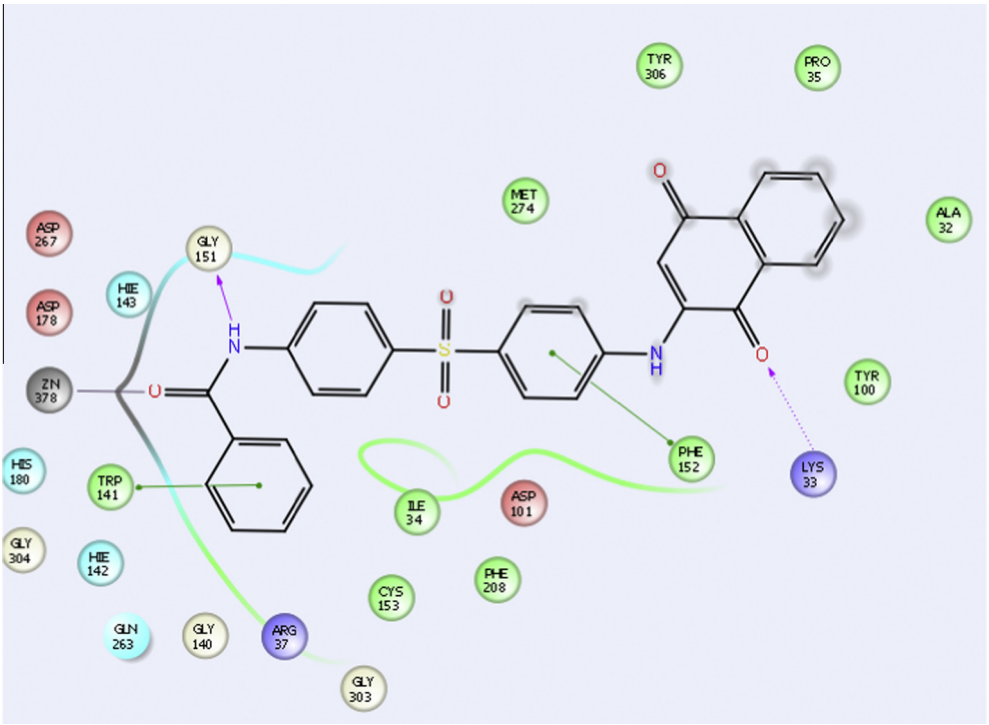


Fig. 5. Docking model structures of compound **2** into the HDAC8 binding pocket.

was separated by filtration. Water was added to the filtrate, the brownish material formed was filtered, washed with hot water (200 mL), dried at 80 °C, and crystallized from 95% ethyl alcohol to give **1** (3.692 g, 91%) as orange crystals.

3.2.2. Method B: [27]

4-Aminophenyl sulfone (2.048 g, 10 mmol) was added to a solution of 1,4-naphthoquinone (1.581 g, 10 mmol) in water (100 mL) and refluxed for 4 h. The reaction mixture was cooled at room temperature and the brownish precipitate was filtered and washed with hot water (200 mL). The precipitate was dried at 80 °C and crystallized from 95% ethyl alcohol to give **1** (1.657 g, 41%) as orange crystals; mp > 300 °C; UV–Vis (acetone): 451.45 nm; IR (KBr): 1294, 1633, 3381, 3475 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.14 (s, 2H), 6.34 (s, 1H), 6.61 (d, 2H, J = 8.8 Hz), 7.53 (d, 2H, J = 8.8 Hz), 7.56 (d, 2H,

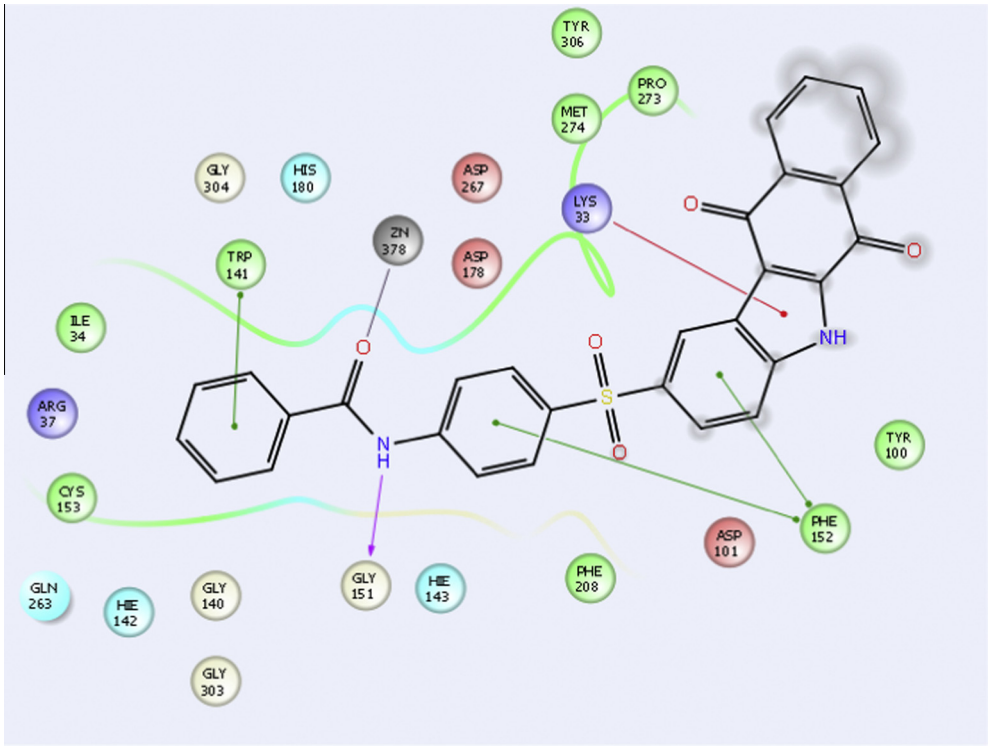


Fig. 6. Docking model structures of compound **2a** into the HDAC8 binding pocket.

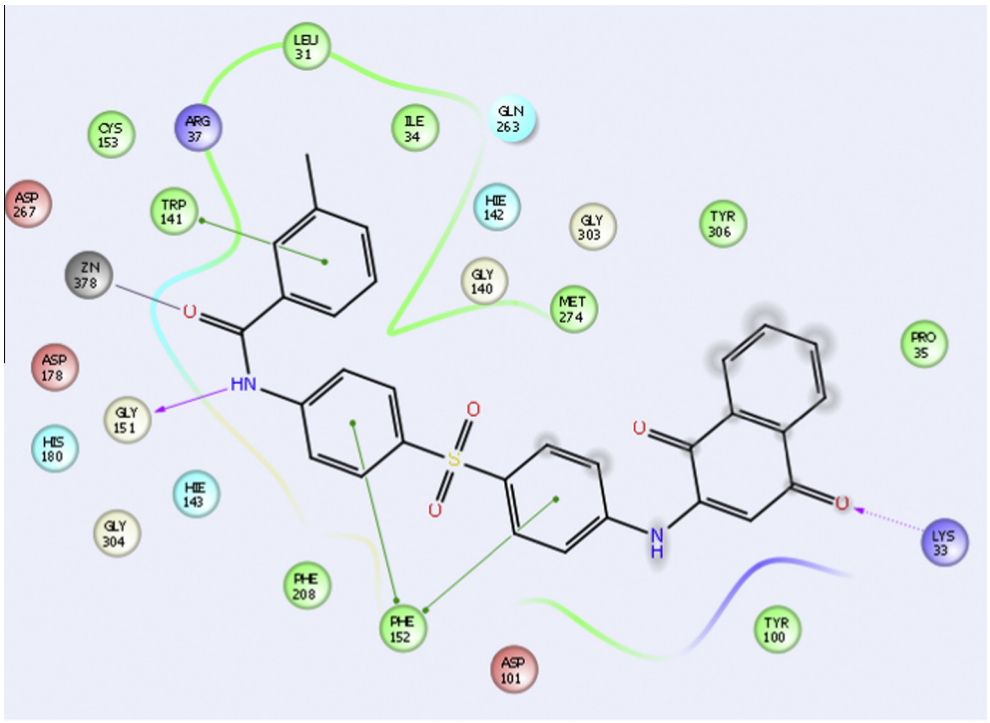


Fig. 7. Docking model structures of compound **3** into the HDAC8 binding pocket.

J = 8.8 Hz), 7.70–7.80 (m, 4H), 7.95 (d, 1H, J = 7.2 Hz), 8.06 (d, 1H, J = 7.3 Hz), 9.41 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 104.4, 112.9, 122.6, 125.2, 125.5, 126.1, 127.8, 129.2, 130.3, 132.2, 132.8, 134.8, 138.2, 142.3, 144.8, 153.5, 181.2, 183.0; MS (EI): m/z 403.49 (M+1, 8%), 257 (80), 180.80 (75), 157.78 (45), 142.78 (60), 122.83 (100).

*3.3. General procedure for synthesis of N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-ylamino) benzenesulfonyl]-phenyl}-aryl benzamides (**2**–**7**)*

Substituted benzoyl chloride (1 mmol) was added to a solution of **1** (0.404 g, 1 mmol) in acetone (100 mL). After refluxing for

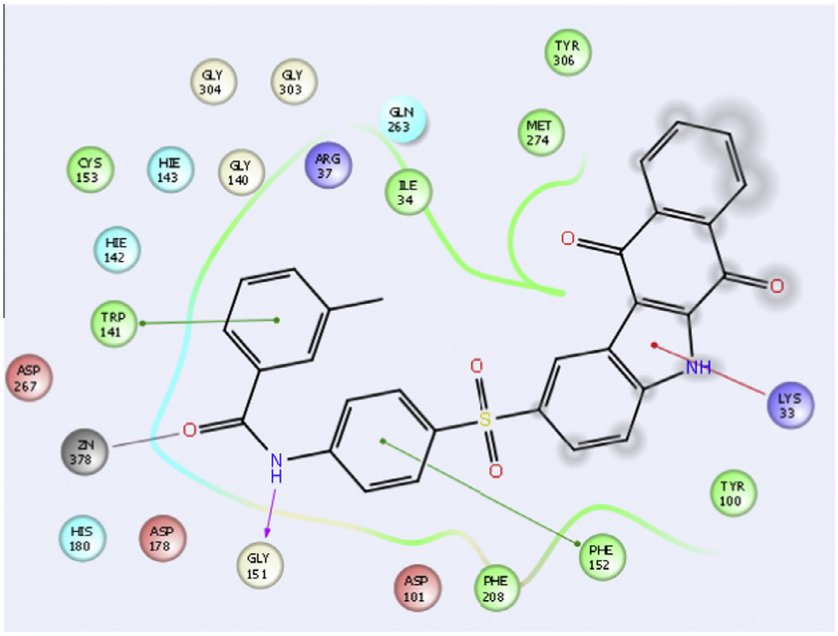


Fig. 8. Docking model structures of compound **3a** into the HDAC8 binding pocket.

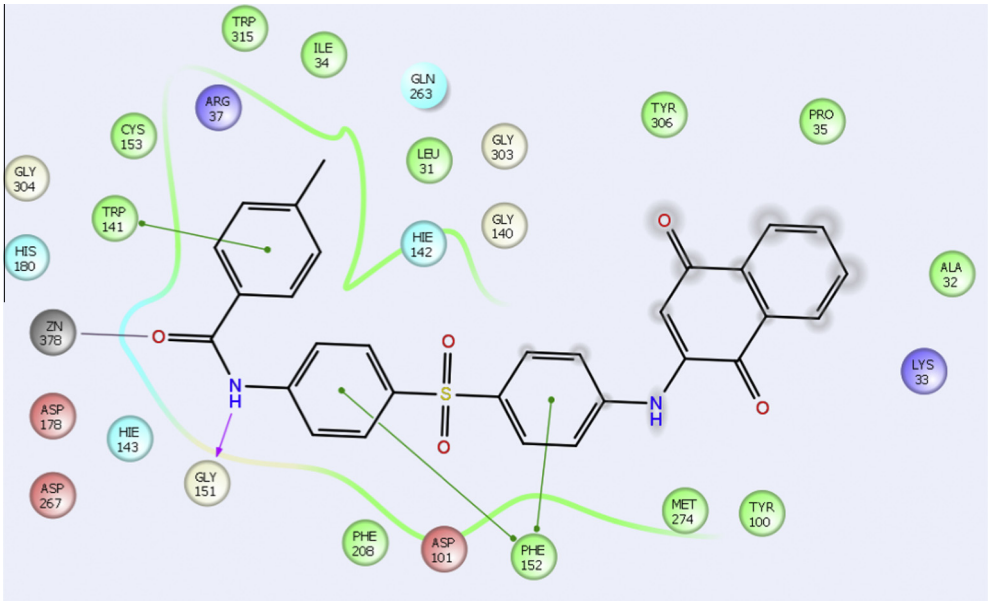


Fig. 9. Docking model structures of compound **4** into the HDAC8 binding pocket.

30 min, the reaction mixture was filtered and concentrated *in vacuo* to give pure samples of **2–7** which required no further purification.

*3.3.1. N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-ylamino)-benzenesulfonyl]-phenyl} benzamide (**2**)*

Orange solid; Reaction time 25 min (0.501 g, 99%); mp > 300 °C; UV–Vis (acetone): 447.36 nm; IR (KBr): 1296, 1631, 1676, 3400 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.39 (s, 1H), 7.54 (d, 2H, J = 6.8 Hz), 7.61–8.07 (m, 15H), 9.45 (s, 1H), 10.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 104.9, 120.2, 122.6, 125.2, 126.2, 127.7, 128.3, 128.4, 128.5, 130.3, 131.9, 132.1, 132.9, 134.2, 134.8, 135.2, 136.2, 143.1, 143.7, 144.6, 166.0, 181.1, 183.0; MS (EI): m/z 508.028 9 (M^+, 12%), 444.63 (55), 300.89 (60), 224.76 (100), 123.07 (60).

*3.3.2. N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-ylamino)-benzenesulfonyl]-phenyl}-3-methyl-benzamide (**3**)*

Red-brown solid; Reaction time 25 min (0.515 g, 99%); mp > 300 °C; UV–Vis (acetone): 445.23 nm; IR (KBr): 1298, 1616, 1680, 2922, 3309 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.08 (s, 3H), 6.40 (s, 1H), 7.36–8.08 (m, 16H), 9.49 (s, 1H), 10.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.3, 105.4, 120.7, 123.1, 125.4, 125.7, 126.7, 126.9, 128.7, 128.8, 128.9, 130.1, 130.8, 131.2,

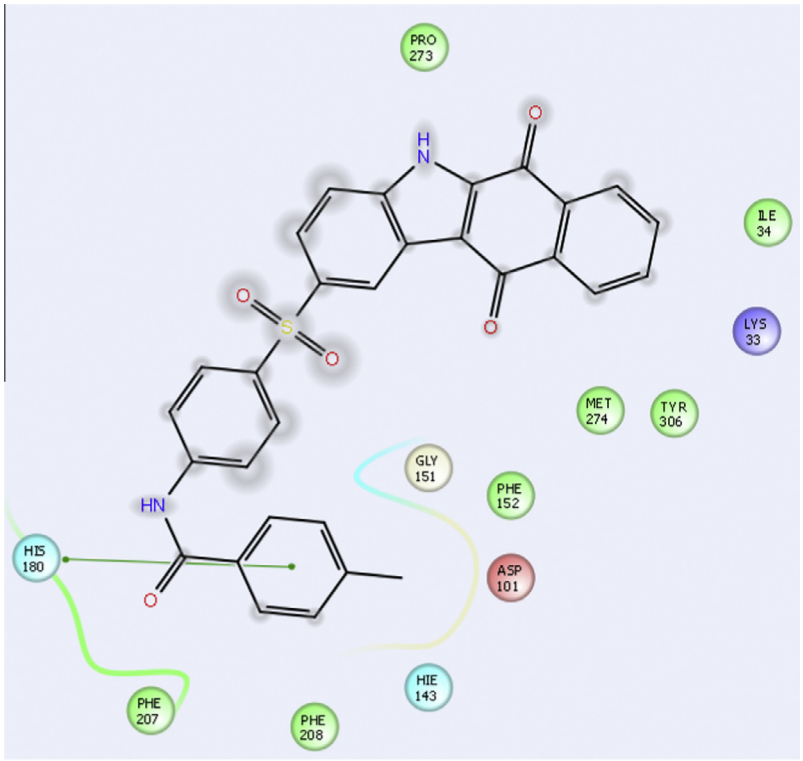


Fig. 10. Docking model structures of compound **4a** into the HDAC8 binding pocket.

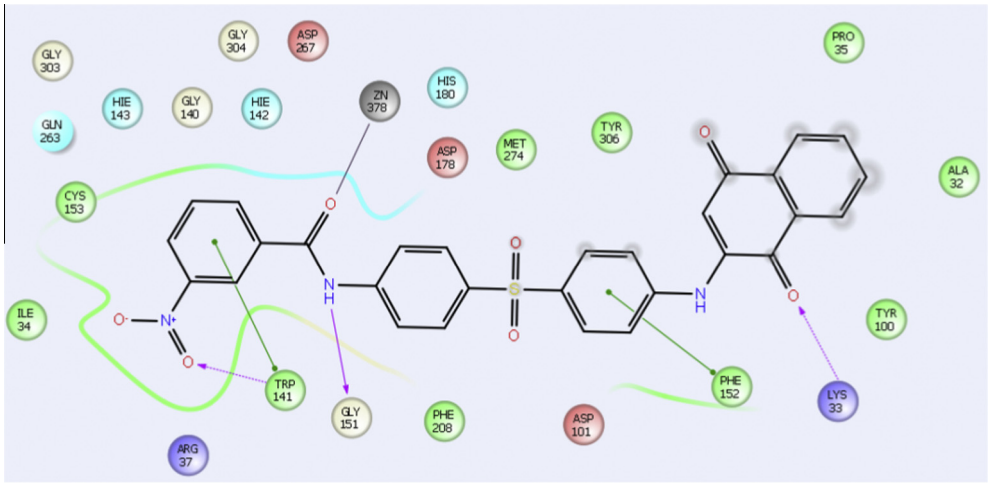


Fig. 11. Docking model structures of compound **5** into the HDAC8 binding pocket.

132.7, 133.0, 133.4, 134.7, 135.7, 138.3, 143.6, 144.3, 167.8, 181.7, 183.6; MS (EI): *m/z* 521.60 (M−1, 15%), 499.17 (25), 457.80 (10), 274.85 (50), 257.85 (25), 175.06 (97), 114.09 (100), 100.07 (76).

*3.3.3. N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-ylamino)-benzenesulfonyl]-phenyl}-4-methyl-benzamide (**4**)*

Crimson red solid; Reaction time 30 min (0.512 g, 98%); mp > 300 °C; UV–Vis (acetone): 448.38 nm; IR (KBr): 1294, 1616, 1680, 2945, 3307, 3360 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H), 6.38 (s, 1H), 7.27 (d, 2H, J = 8.0 Hz), 7.32 (d, 2H, J = 8.0 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.77–8.06 (m, 10H), 9.45 (s, 1H), 10.53 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.9, 104.8, 120.1, 122.6, 125.2, 126.1, 127.8, 128.3, 128.9, 129.2, 130.3, 131.3, 132.1, 132.9, 134.8, 135.1, 136.3, 142.1, 143.1, 143.8, 144.6, 165.8, 181.1, 183.0; MS (EI): *m/z* 521.54 (M−1, 45%), 456.86 (15), 250.75 (80), 184.42 (100).

*3.3.4. N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-ylamino)-benzene sulfonyl]-phenyl}-3-nitro-benzamide (**5**)*

Red-brown solid; Reaction time 25 min (0.545 g, 99%); mp > 300 °C; UV–Vis (acetone): 447.36 nm; IR (KBr): 1274, 1348, 1529, 1616, 1687, 3412 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.40 (s, 1H), 7.63 (d, 2H, J = 7.2 Hz), 7.82 (d, 2H, J = 7.2 Hz), 7.85–8.06 (m, 7H), 8.34 (d, 1H, J = 7.3 Hz), 8.40 (d, 1H, J = 7.3 Hz), 8.47 (d, 1H, J = 7.8 Hz), 8.62 (s, 1H), 9.50 (s, 1H), 10.96 (s, 1H), 13.71 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.4, 121.0, 123.1, 123.2, 124.1, 125.7, 126.7, 127.7, 128.9, 129.1, 130.7, 130.8, 131.2, 132.6, 132.9, 133.4, 135.3, 135.8, 136.1, 143.8, 145.1,

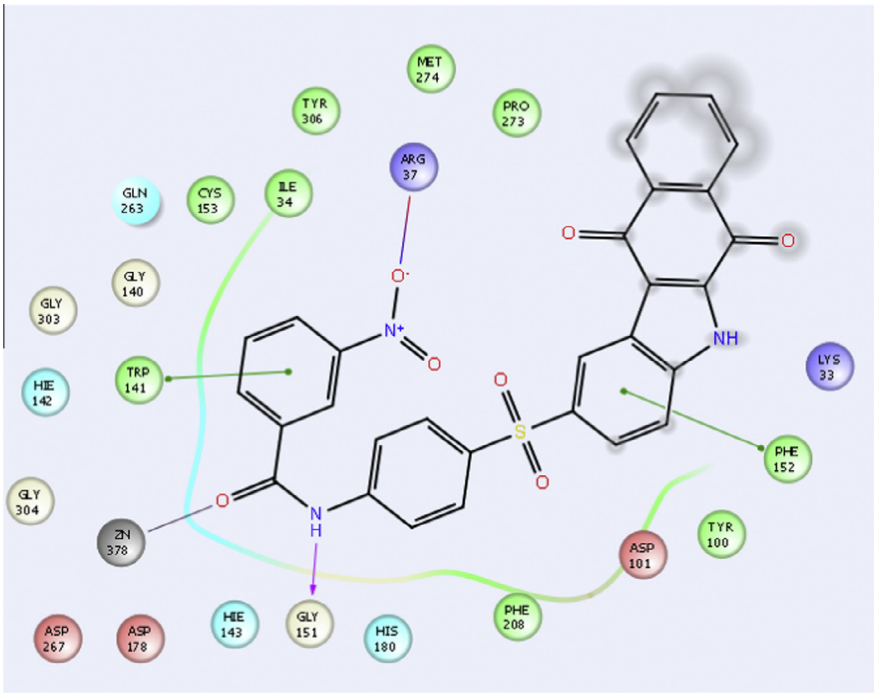


Fig. 12. Docking model structures of compound **5a** into the HDAC8 binding pocket.

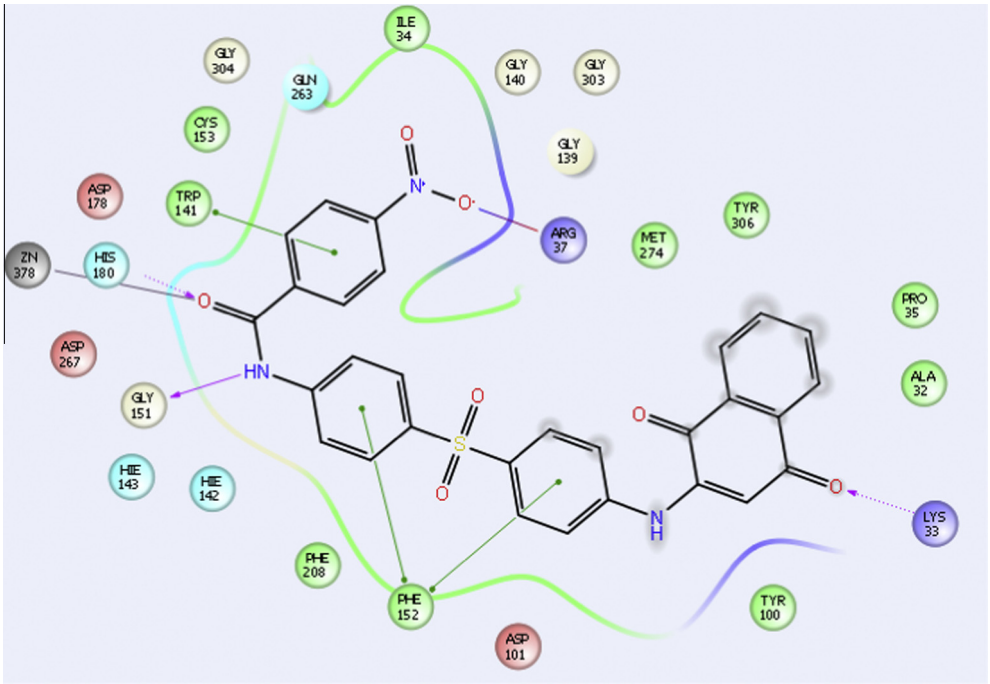


Fig. 13. Docking model structures of compound **6** into the HDAC8 binding pocket.

148.2, 164.4, 181.6, 183.6; MS (EI): *m/z* 553.12 (M^+, 13%), 507.98 (15), 440.31 (38), 366.39 (100), 293.45 (41), 232.44 (40).

*3.3.5. N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-yl amino)-benzene sulfonyl]-phenyl}-4-nitro-benzamide (**6**)*

Orange solid; Reaction time 25 min (0.543 g, 98%); mp > 300 °C; UV–Vis (acetone): 453.49 nm; IR (KBr): 1273, 1350, 1529, 1614, 1680, 3248, 3315 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.39 (s, 1H), 7.62 (d, 2H, J = 8.0 Hz), 8.79 (t, 1H, J = 7.8 Hz), 7.86 (t, 1H, J = 7.8 Hz), 7.95 (d, 2H, J = 8.0 Hz), 7.98 (d, 2H, J = 8.0 Hz), 8.02 (d, 2H, J = 8.0 Hz), 8.06 (d, 1H, J = 7.8 Hz), 8.14 (d, 1H, J = 7.8 Hz), 8.18 (d, 2H, J = 8.0 Hz) 8.37 (d, 2H, J = 8.0 Hz), 9.46 (s, 1H), 10.92 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 104.9, 120.4, 122.6, 123.5, 125.2, 126.2, 126.2, 128.4, 128.6, 130.3, 132.1, 132.9, 134.3, 135.3, 135.6, 135.8, 136.1, 143.2, 144.6, 149.3, 164.4, 181.1, 183.0; MS (EI): *m/z* 551.85 (M−2, 10%), 528.20 (30), 510.51 (25), 268.46 (75), 252.45 (98), 191.36 (100), 177.40 (52).

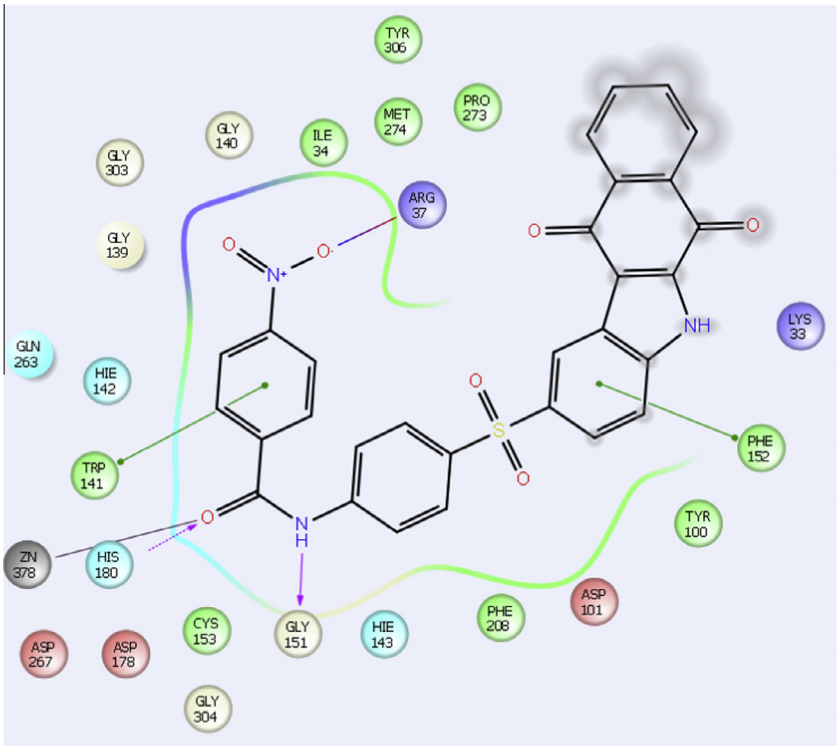


Fig. 14. Docking model structures of compound **6a** into the HDAC8 binding pocket.

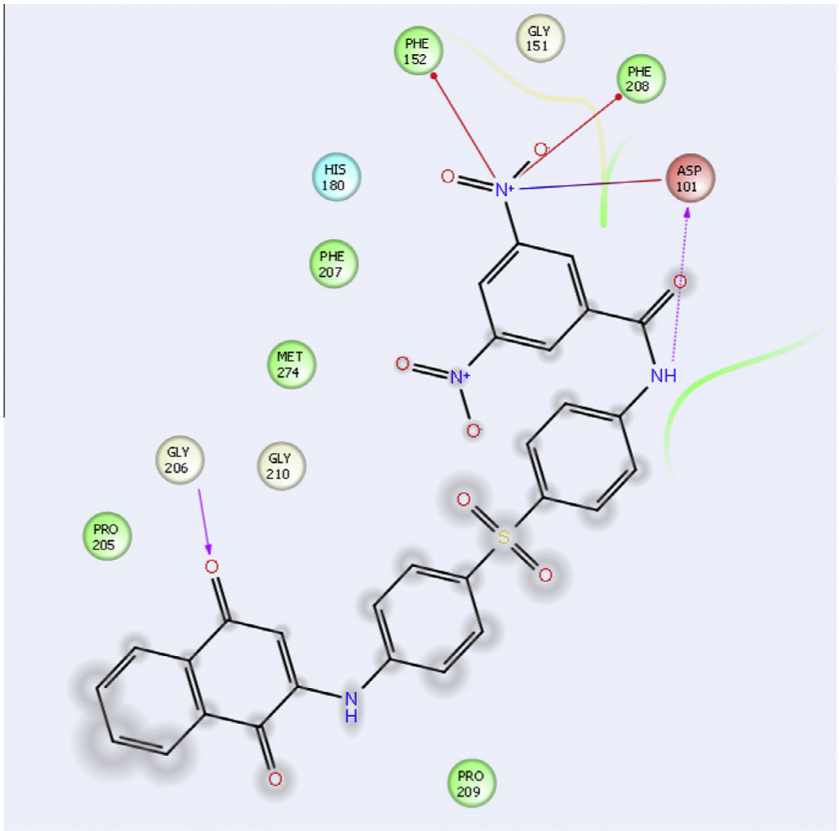


Fig. 15. Docking model structures of compound **7** into the HDAC8 binding pocket.

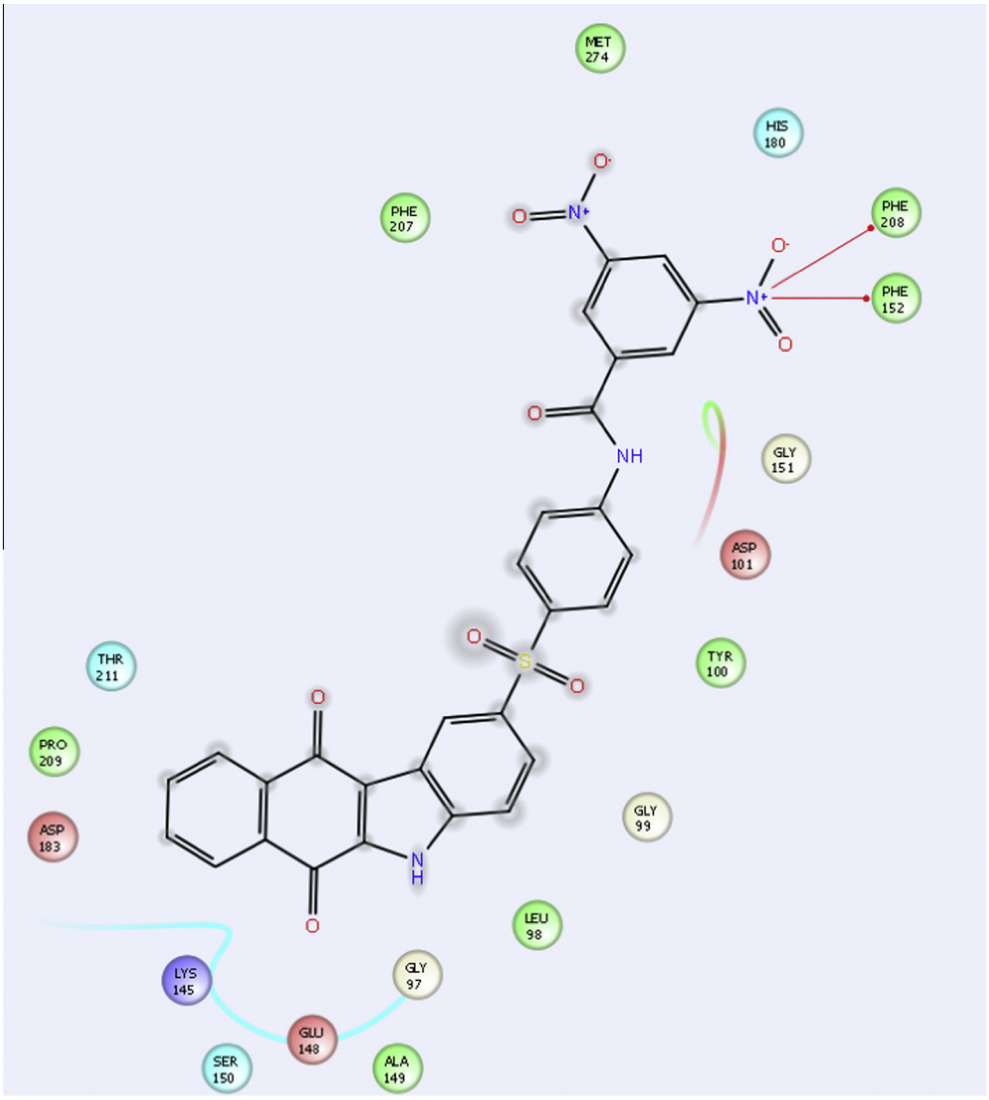


Fig. 16. Docking model structures of compound **7a** into the HDAC8 binding pocket.

*3.3.6. N-{4-[4-(1,4-dioxo-1,4-dihydro-naphthalene-2-yl amino)-benzene sulfonyl]-phenyl}-3, 5-dinitro-benzamide (**7**)*

Red-brown solid; Reaction time 30 min (0.589 g, 98%); mp > 300 °C; UV–Vis (acetone): 446.12 nm; IR (KBr): 1271, 1346, 1541,1624, 1691, 3334, 3400 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.39 (s, 1H), 7.62 (d, 2H, J = 8.8 Hz), 7.78 (t, 1H, J = 6.8 Hz), 7.82 (t, 1H, J = 6.8 Hz) 7.93 (d, 2H, J = 7.2 Hz), 7.93–8.05 (m, 8H), 9.17 (s, 1H), 9.45 (s, 1H), 11.16 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 104.9, 120.7, 121.4, 122.6, 125.2, 126.1, 128.1, 128.6, 130.3, 132.1, 132.9, 134.8, 134.8, 136.2, 136.8, 142.8, 143.2, 144.6, 148.0, 148.2, 161.9, 181.1, 183.0; MS (EI): *m/z* 596.51 (M−2, 55%), 566.45 (20), 537.60 (25), 473.23 (15), 399.43 (100), 382.51 (46), 218.53 (45).

*3.4. General procedure for synthesis of 2-(4-amino-benzosulfonyl)-5H-benzo [b]carbazole-6,11-diones (**1a**–**7a**)*

3.4.1. Method C: [22,23]

Mixture of **1**–**7** (0.5 mmol) in glacial acetic acid (60 mL) and palladium (II) acetate (0.112 g, 0.5 mmol) were refluxed for 2 h and the reaction mixture was cooled at room temperature and poured into ice cold water. The precipitate was filtered, dried at 60 °C and crystallized from acetone to give **1a–7a**.

*3.4.2. 2-(4-amino-benzosulfonyl)-5H-benzo [b]carbazole-6, 11-dione (**1a**)*

Yellow solid; Reaction time 2 h (0.150 g, 75%); mp > 300 °C; UV–Vis (acetone): 278.71 nm; IR (KBr): 1288, 1629, 1651, 3384, 3478 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 6.13 (s, 2H), 6.60 (d, 2H, J = 8.8 Hz),, 7.55 (d, 2H, J = 8.8 Hz), 7.70 (d, 1H, J = 8.8 Hz), 7.80–7.90 (m, 3H), 8.09 (d, 1H, J = 5.2 Hz), 8.10 (d, 1H, J = 5.2 Hz), 8.60 (s, 1H), 13.4 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.5, 118.4, 121.9, 123.6, 125.0, 126.2, 126.7, 129.8, 130.3, 133.0, 134.0, 134.3, 135.0, 139.1, 139.8, 140.0, 151.0, 154.0, 177.9, 180.7; MS (EI): *m/z* 402.40 (M^+, 5%), 342.75 (8), 250.87 (50), 205.06 (98), 162.06 (100), 117.10 (60), 75.10 (45).

*3.4.3. N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-benzamide (**2a**)*

Light yellow solid; Reaction time 2 h (0.195 g, 77%); mp > 300 °C; UV–Vis (acetone): 278.79 nm; IR (KBr): 1244, 1589, 1668, 3375 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 7.58 (t, 2H, J = 8.0 Hz), 7.60 (t, 1H, J = 8.0 Hz), 7.76 (d, 1H, J = 8.8 Hz),

Table 1
Molecular docking data of compounds **1**–**7**, **1a**–**7a.**

Compounds			Molecular docking			
	Glide score	Glide energy (kcal/mol)	E model score (kcal/mol)	No. of hydrogen bonds	Bond length (ÅA)	XP H bond
1	−4.38	−38.55	−50.17	Hydrophophic interaction	–	−0.00 e^+
2	−8.52	−67.78	−70.06	2 (GLY 151, LYS 33)	1.915 (C–O–H), 2.152 (N–H–O)	−1.04
3	−9.06	−64.78	−73.41	2 (GLY 151, LYS 33)	1.855 (C–O–H), 2.070 (N–H–O)	−1.27
4	−8.25	−64.04	−45.50	1 (GLY 151)	1.829 (C–H–O)	−0.55
5	−8.17	−73.60	−68.17	3 (GLY 151, LYS 33, TRP 141)	1.857 (C–H–O), 2.237 (N–H–O), 2.463 (N–H–O)	−0.94
6	−8.59	−64.52	−65.50	3 (GLY151, LYS 33, HIS 180)	1.821 (C–O–H), 2.029 (N–H–O), 1.917 (N–H–O)	−1.32
7	−3.42	−43.17	−65.34	2 (ASP 101, GLY 206)	1.962 (C–O–H), 2.019 (N–H–O)	−0.99
1a	−4.35	−36.57	−46.54	1 (GLY 151)	2.060 (C–H–O)	−0.90
2a	−8.35	−65.44	−58.16	1 (GLY 151)	1.763 (C–O–H)	−0.95
3a	−8.96	−63.87	−72.72	1 (GLY 151)	1.852 (C–O–H)	−0.91
4a	−3.74	−36.27	−53.67	Hydrophophic interaction	–	−0.00 e^+
5a	−8.03	−71.89	−84.18	1 (GLY 151)	1.880 (C–H–O)	−0.93
6a	−6.55	−59.68	−65.27	2 (HIS 180, GLY 151)	1.814 (C–H–O)	0.97
7a	−2.97	−50.69	−71.02	Hydrophophic interaction	–	0.00 e^+
SAHA	−9.42	−63.82	−84.04	PHE 208	1.862 (N–H–O), 1.880 (N–H–O)	−1.43

7.81–7.92 (m, 5H), 7.90 (d, 2H, *J* = 8.2 Hz), 8.03 (d, 2H, *J* = 8.2 Hz), 8.10–8.16 (m, 2H), 8.76 (d, 1H, *J* = 1.2 Hz), 10.61 (s, 1H), 13.47 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.3, 117.9, 120.3, 122.2, 123.2, 123.3, 124.8, 126.2, 127.7, 128.4, 131.9, 132.4, 132.5, 133.5, 133.7, 134.2, 134.5, 135.3, 136.8, 139.4, 139.8, 143.7, 166.0, 177.4, 180.2; MS (EI): *m/z* 505.84 (M^+, 5%), 491.09 (35), 474.60 (10), 450.66 (15), 259.18 (20), 218.31 (60), 198.36 (45), 125.41 (35), 81.36 (100).

*3.4.4. N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-3-methyl-benzamide (**3a**)*

Light yellow solid; Reaction time 2 h (0.199 g, 77%); mp > 300 °C; UV–Vis (acetone): 339.39 nm; IR (KBr): 1244, 1589, 1668, 2922, 3255 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.38 (s, 3H), 7.40 (m, 2H, *J* = 7.8 Hz), 7.70–8.20 (m, 12H), 8.77 (s, 1H), 10.59 (s, 1H), 13.54 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.3, 115.8, 120.7, 122.7, 125.4, 126.7, 128.7, 128.8, 128.9, 132.4, 133.0, 133.5, 134.0, 134.2, 134.7, 135.0, 135.8, 137.3, 138.2, 139.9, 140.3, 142.6, 143.2, 166.6, 178.1, 181.0; MS (EI): *m/z* 518.45 (M−2, 15%), 496.07 (15), 477.41 (20), 300.78 (12), 226.92 (35), 171.95 (100), 110.98 (75), 96.96 (52).

*3.4.5. N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-4-methyl-benzamide (**4a**)*

Brick red color solid; Reaction time 2 h (0.201 g, 78%); mp > 300 °C; UV–Vis (acetone): 346.67 nm; IR (KBr): 1242, 1651, 1666, 2922, 3352 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.36 (s, 3H), 7.31 (d, 2H, *J* = 8.0 Hz), 7.75–8.16 (m, 12H), 8.75 (s, 1H), 10.51 (s, 1H), 13.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.9, 115.3, 117.9, 120.2, 122.2, 123.1, 124.8, 126.2, 127.8, 128.3, 128.9, 131.3, 131.5, 132.4, 133.5, 133.7, 134.5, 135.2, 136.8, 139.4, 139.8, 142.1, 143.8, 165.8, 177.4, 180.2; MS (EI): *m/z* 521.85 (M+1, 25%), 471(75), 250.37 (30), 184 (100), 81.79 (65).

*3.4.6. N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-3-nitro-benzamide (**5a**)*

Pale yellow solid; Reaction time 2 h (0.212 g, 77%); mp > 300 °C; UV–Vis (acetone): 277.58 nm; IR (KBr): 1251, 1350, 1525, 1591, 1670, 3257 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.70–8.20 (m, 15H) 10.94 (s, 1H), 13.55 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 106.9, 119.1, 122.9, 123.2, 124.5, 125.6, 125.6, 125.3, 125.1, 126.4, 127.1, 129.1, 131.2, 132.4, 132.5, 132.9, 133.2, 134.1, 134.4, 134.9, 136.0, 137.1, 139.2, 146.1, 163.4, 180.2, 181.0; MS (EI): *m/z* 550.65 (M−1, 15%), 536.40 (30), 507.28 (10), 437.65 (10), 408.63 (15), 351.84 (38), 289.86 (95), 233.83 (100), 222.08 (18).

*3.4.7. N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-4-nitro-benzamide (**6a**)*

Yellow solid; Reaction time 2 h (0.215 g, 78%); mp > 300 °C; UV–Vis (acetone): 258.18 nm; IR (KBr): 1319, 1348, 1529, 1591, 1678, 3437 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.64–8.39 (m, 15H), 9.81 (s, 1H), 10.80 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 117.1, 119.0, 122.0, 122.1, 123.5, 124.6, 126.1, 126.3, 126.8, 127.8, 128.4, 129.0, 131.0, 132.1, 132.7, 132.8, 133.4, 135.1, 135.2, 136.4, 138.0, 140.0, 163.1, 180.7, 182.4; MS (EI): *m/z* 548.56 (M−3, 5%), 527.29 (20), 509.61 (20), 251.56 (80), 190.52 (100), 164.77 (18).

*3.4.8. N-[4-(6,11-dioxo-6,11-dihydro-5H-benzo[b]carbazole-2-sulfonyl)-phenyl]-3,5-dinitro-benzamide (**7a**)*

Yellow solid; Reaction time 2 h (0.228 g, 77%); mp > 300 °C; UV–Vis (acetone): 269.09 nm; IR (KBr): 1244, 1344, 1535, 1629, 1660, 3300 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.75–8.15 (m, 10H), 8.76 (d, 1H, *J* = 1.2 Hz), 8.98 (t, 1H, *J* = 2.0 Hz), 9.10 (d, 2H,

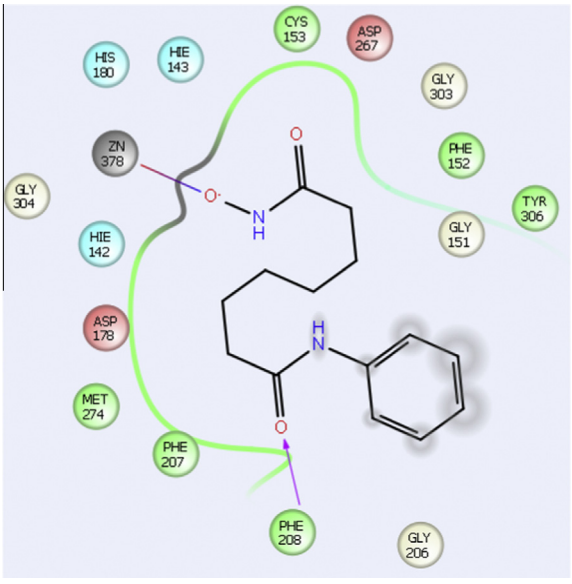


Fig. 17. Docking model structures of compound **SAHA** into the HDAC8 binding pocket.

J = 2.0 Hz) 11.14 (s, 1H), 13.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.3, 117.9, 120.8, 212.4, 211.3, 123.2, 124.8, 126.1, 128.1, 128.5, 132.4, 133.5, 133.7, 134.4, 136.3, 136.5, 136.8, 139.4, 139.8, 142.8, 148.0, 148.9, 161.9, 177.4, 180.2; MS (EI): m/z 595.42 (M−1, 35%), 562.58 (15), 543.04 (40), 407.72 (45), 386.74 (60), 328.63 (100), 249.72 (75), 214.68 (32).

3.5. Measurement of cytotoxicty

To evaluate the cytotoxic property of the synthesized quinone derivatives, the MTT assay was carried out [24]. 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 between 5 and 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 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 (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, CA, USA). Data were collected for three replicates each and used to calculate the mean. The percentage inhibition was calculated, from the data, using the formula given below, and IC_{50} values were calculated using nonlinear regression analysis.

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

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

3.6. Molecular docking studies

To understand the interaction of all the synthesized molecules (**1–7**, **1a–7a**) with HDAC8, the molecular docking studies were performed using the GLIDE program [28] (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. SAHA (Fig. 17) (PDB ID: 1T69) was selected for docking studies as a reference sample and was prepared for docking through protein preparation wizard. Structures of **1–7**, **1a–7a** 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.

4. Conclusion

A new series of carbazole-6,11-dione derivatives have been synthesized and characterized fully by UV–Vis, FT-IR, ^{1}H, ^{13}C NMR and mass spectral analyses. *In vitro* cytotoxicity study of all the synthesized compounds were carried out and reported. It is found that compounds **1a**, **4a** and **7a** exhibited good cytotoxicity against human cervical cancer cell line (SiHa). Molecular docking of compound **3** was the one with the best glide and E model score of −9.06 and −73.41, respectively which is close to the glide score of SAHA (standard). In all docked molecules, the compound **7a** exhibits least glide and E model score of −2.97 and −71.02 respectively.

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