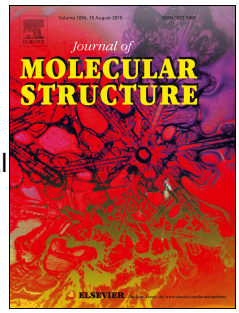
Accepted Manuscript



Design and synthesis of novel sulphamide tethered quinazolinone hybrids as potential antitumor agents

Ramineni Venkatesh, Suresh Kasaboina, Nishant Jain, Sridhara Janardhan, Uma Devi Holagunda, Lingaiah Nagarapu

PII: S0022-2860(18)31532-1

DOI: https://doi.org/10.1016/j.molstruc.2018.12.098

Reference: MOLSTR 26035

To appear in: *Journal of Molecular Structure*

Received Date: 23 August 2018

Revised Date: 26 November 2018

Accepted Date: 23 December 2018

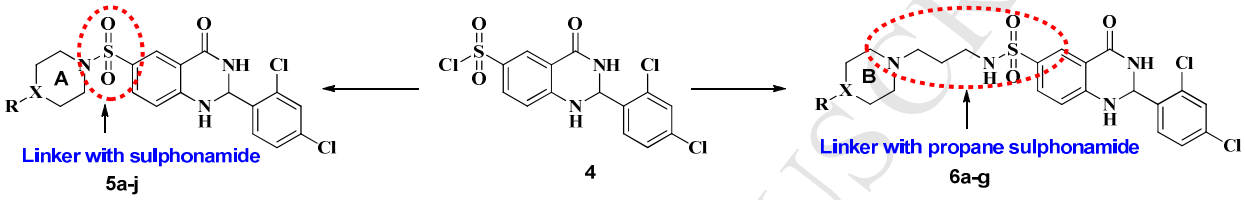
Please cite this article as: R. Venkatesh, S. Kasaboina, N. Jain, S. Janardhan, U.D. Holagunda, L. Nagarapu, Design and synthesis of novel sulphamide tethered quinazolinone hybrids as potential antitumor agents, *Journal of Molecular Structure* (2019), doi: https://doi.org/10.1016/j.molstruc.2018.12.098.

Graphical Abstract

Design and synthesis of novel sulphamide tethered quinazolinone hybrids as potential antitumor agents

Ramineni Venkatesh[a], Suresh Kasaboina[a], Nishant Jain[b], Sridhara Janardhan[c], Uma Devi Holagunda[a],Lingaiah Nagarapu[a*]

A series of novel sulphamide tethered quinazolinone derivatives were efficiently synthesized and evaluated for antitumor activity against four cancer cell lines.



Design and synthesis of novel sulphamide tethered quinazolinone hybrids as potential antitumor agents

Ramineni Venkatesh[a], Suresh Kasaboina[a], Nishant Jain[b], Sridhara Janardhan[c], Uma Devi Holagunda[a], Lingaiah Nagarapu[a*]

[a]Fluoro-Agrochemicals Division-II, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad- 500007, India.

[b]Center for Chemical Biology, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500007, India.

[c]Center for Molecular Modeling, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India.

*Corresponding author: e-mail: lnagarapuiict@yahoo.com/nagarapu@csiriict.in
Tel: +91 40 27191509, Fax; *++ 91 40 27193382. IICT/Pubs./2018/049*

Abstract: In an attempt to develop potential and selective antitumor agents, a series of novel sulphamide tethered quinazolinone hybrids were efficiently synthesized and evaluated for antitumor activity against four cancer cell lines such as HeLa (cervical), MDA-MB-231 (breast), PANC-1 (pancreatic), and A549 (lung) *in vitro*. All the compounds (**5a-j**, **6a-g**) exhibited significant anti-proliferative activity with GI_{50} values ranging from 0.045 to 6.94 μM, while compound **10c** showed potent activity against all the cell lines (He La, MDA-MB-231, PANC-1 and A549) with GI_{50} values ranging from 0.09 to 0.21 μM. We have explored the binding mode and key active site interactions in HDAC8 and EHMT2 proteins. The docking results are complementary to the experimental results.

Keywords: Quinazolinones scaffolds, sulphamides, antitumor activity, docking studies.

Introduction

The potent antitumor activities and clinical applications of quinazolinones scaffold containing alkaloids have attracted worldwide. Their parent representative analogues serves as an excellent lead drug in anticancer research because of its potent antitumor activity [1,2]. Quinazolinones have been reported to have diverse range of biological activities such as anticancer, anti-HIV, anti tubercular, diuretic, anti-inflammatory, anticonvulsant, antiviral, anti malarial, antibacterial, Antifungal activity and antihypertensive agents [3-10]. Recently, literature reports revealed that the importance of sulphones and their derivatives in the medicinal chemistry due to their use as precursors in the synthesis of various biologically active heterocyclic compounds. They are used as significant pharmacological activities such

as antifungal, antibacterial, anti cancer and antimicrobial [11-16] activities. Chemically, sulfonyl and sulphanamide functional groups are well established as activating moiety in a number of intermediates for the construction of C-S and C-N hetero atoms bond formation during organic transformations. Recently, they have been utilized in total synthesis and in the generation of database of functionalized compounds. Moreover, these moieties have potential to stabilize carnations, radicals and facilitate conjugate addition by activation of the olefins with Lewis acids. In past decades, numerous quinazolinones embedded natural products have been identified, such as cytotoxic alkaloids Luotonin A, Camptothecin (CPT), Aromathecin, Rosettacin, 22-Hydroxyacuminatine, Rutaecarpineetc, whose derivatives are clinically proven anticancer agents [17-25]. Additionally, some therapeutic agents are in the market and some are in clinical trials for the treatment of cancer [26]. A literature survey revealed that modification on quinazolinone pharmacophore may result increase in its biological potencies. Structural variations bring about new physical and biological properties. The molecular modification of a promising lead compound is still a major line of approach for the discovery of new drugs. As a part of synthesis of new class of quinazolinones derivatives, we have introduced sulphamide group with several secondary amines which might be a potential tumor growth inhibitor. Recently, in an on-going programme to discover and develop potential new anticancer agents, we have identified several classes of molecules as novel tumor growth inhibitors [27-31]. In an attempt to design new anticancer agents, we discovered a novel series of quinazolinone derivatives (**Fig. 1**) to elicit combined antitumor efficacy/cytotoxicity against different cancer cell lines *in vitro.* The computational techniques play a vital role in lead molecule identification and its optimization. Both structure based (homology modeling, docking) and ligand based (pharmacophore, QSAR) drug design approaches have been used for the design of better ligands in order to enhance potency, selectivity, pharmacokinetic parameters. Hence, in the current study, we have applied both ligand based and structure based techniques on a series of quinazolinone hybrids in both EHMT2 and HDAC8 proteins. The crystal structures of EHMT2 and HDAC8 are known and hence we have downloaded from the protein databank for further analysis. The docking studies have been performed to understand the key active site residues of most potent quinazolinone series of compounds in all EHMT2 and HDAC8 proteins and explored all probable binding modes. Ligand based pharmacophore model will be helpful in identifying drug-like features for responsible for better ligand binding. The pharmacophore models provide the hypothetical active site and can be used for virtual screening [32-35] to obtain diverse chemical classes of new compounds. The identified structural features from ligand

based and structure based approaches could help in rational design of quinazolinone series of compounds with enhanced potency in EHMT2 and HDAC8 proteins.

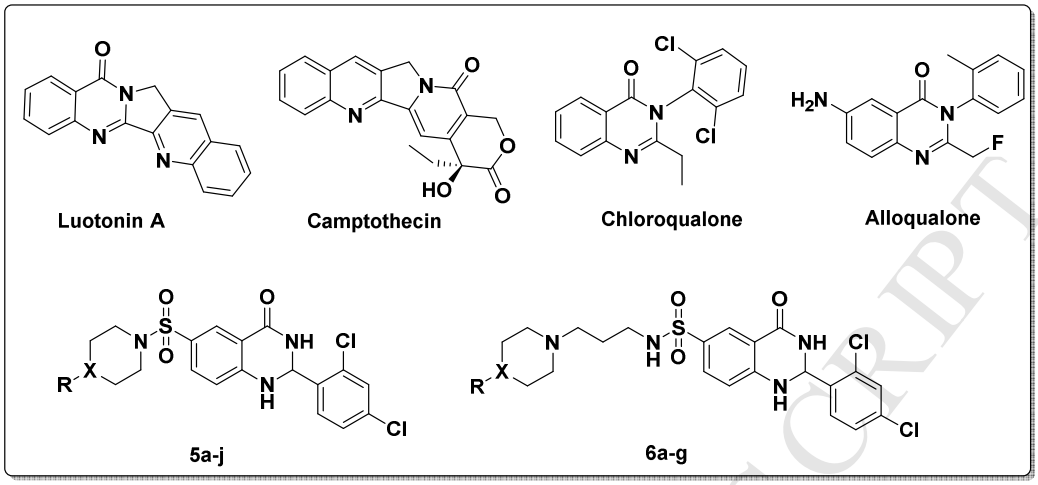
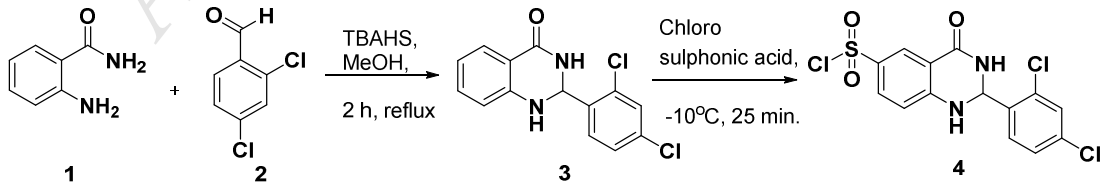


Figure 1. Structures of Luotonin A, Camptothecin, Chloroqualone, Alloqualone and quinazolinone derivatives.

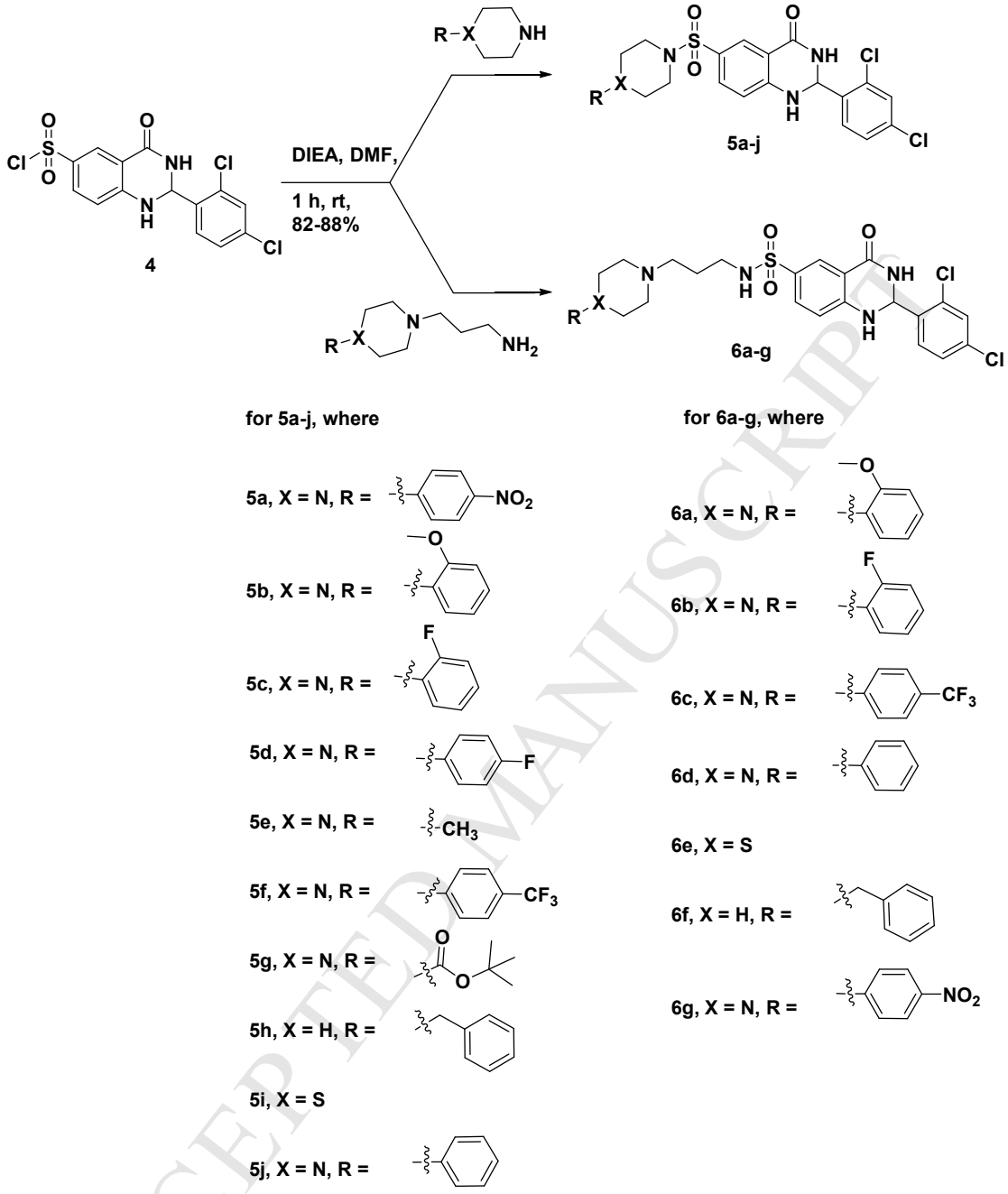
Results and discussion:

Chemistry

The synthesis of 2-(2,4-dichlorophenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (**4**) was done by the condensation of anthranilamide (**1**) with 2,4-dichloro benzaldehyde (**2**) and a catalytic amount of tetrabutyl ammonium hydrogensulphate (TBAHS) in methanol under reflux temperature for 2 h followed by the treatment with chlorosulphonicacid at -10 ^{o}C for 25min to produce compound (**4**) in good yield (Scheme 1). The synthesis of sulphamide tethered quinazolinone-piperzine hybrids (**5a-j, 6a-g**) have been achieved in moderate to excellent yields by the coupling of compound **4** with *N*-phenyl piperzine derivatives and 3-(4-phenylpiperazin-1-yl)propan-1-amine derivatives using diisopropylethyl amine (DIEA) in DMF under room temperature for 1h as shown in Scheme 3.



Scheme-1. Synthesis of compound **4**.



Scheme-2. Synthesis of compounds **5a-j, 6a-g.**

Pharmacology:

Cytotoxicity of all the compounds were evaluated against human cancer cell lines He La (cervical), MDA-MB-231 (breast), PANC-1 (pancreatic), and A549 (lung) by the standard MTT assay method [36-38]. The various human tumor cell growth inhibitor potential was determined as described [39] The compounds were tested for an advanced assay against these four human cancer cell lines at five different concentrations (0.01, 0.1, 1, 10, 100 μM). GI_{50}

(growth inhibitory activity) was calculated and these values corresponded to the concentration of the compound causing 50% decrease in the net cell growth as compared to the standard drugs, Doxorubicin, Nocadazole, Colchicine, Cambretostatin (CA4) and Paclitaxel (**Table 2**). Results were calculated for each of these parameters if the level of activity was reached; however, if the effect was not achieved, the value was expressed as greater or less than the maximum or minimum concentration tested.

Based on Table 2, all the synthesized compounds showed significant cancer cell growth inhibition with GI_{50} values ranging from 0.045 to 6.94 μM. In particular, the compounds **5a, 5c, 5f, 5g, 5i, 6c, 6e, 6f,** and **6g** showed promising anti-proliferative activity against the four human cancer cell lines. Among them, compounds **5c** showed potent anti-proliferative activity against all the four human cancer cell lines with GI_{50} values ranging from 0.09 to 0.21 μM. The compounds **5i** and **6f** showed potent anti-proliferative activity against the three human cancer cell lines He La, MDA-MB-231, and A549 with GI_{50} values ranging from 0.045 to 0.35 μM. The compounds **6e** showed significant anti-proliferative activity against the two human cancer cell lines He La, and PNAC-1 with GI_{50} values 0.44 and 0.48 μM respectively. The selective anti-proliferative activity of compounds are as follows, **5a** against He La (GI_{50} 0.67 μM), **5f** against MDA-MB-231 (GI_{50} 0.35 µM), **5g** against MDA-MB-231 (GI_{50} 0.29 μM), **6c** against HeLa (GI_{50} 0.31 μM) and **6g** against MDA-MB-231 (GI_{50} 0.61 μM). The effect of various substituents on the sulphonamide moiety was examined. The structure-activity relationship (SAR) study revealed that not only the substituent on sulphamide but also the sulphamide along with propyl linker is required for inducing the anti-proliferative activity against these cancer cell lines. The substituents on sulphamide such as *o*-fluorophenyl (**5c**), thiopiperidine (**5i**) and propyl linker substitution on thiopiperidine (**6e**) and benzyl (**6f**) were associated with a significant increase in the growth inhibitory effect against He La, MDA-MB-231, PANC-1 and A549 human cancer cell.

Molecular modeling:

Pharmacophore modeling

In order to identify the essential structural features of the ligand responsible for its potency, we have generated a ligand based pharmacophore model from a dataset comprising of (**5a-j, 6a-g**) quinazolinone compounds using A549 cell-line activity. A total of 428 conformations thus obtained were used to generate pharmacophore hypotheses. The series of compounds having maximum variation in their growth inhibition (pGI_{50}: 5.34 to 7.34). The common pharmacophore hypothesis (CPH) was obtained based 6 highly active compounds

($pIC_{50} \geq 6$) from the dataset and then inactive molecules ($pIC_{50} < 6$) were aligned with it. The identified best CPH consists of one acceptor (A), one donor (D), one hydrophobic (H) and two aromatic (R) features i.e ADHRR. Alignment of the set of active compounds from the dataset using pharmacophore hypothesis (ADHRR) is shown in Figure 2.

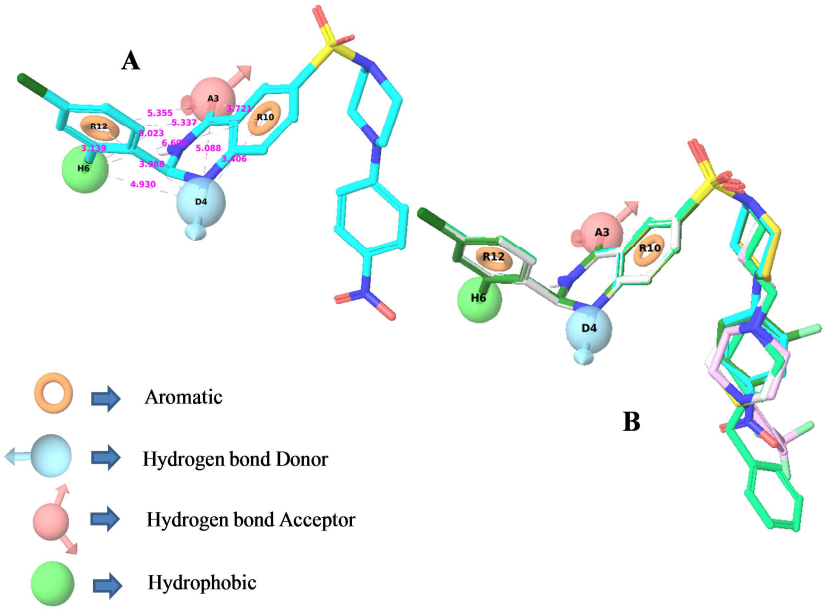


Figure 2. A: Common pharmacophore hypothesis (ADHRR) and its pharmacophoric distances (Å) are shown in pink colour. **B**: Alignment of active data set compounds on ADHRR.

Molecular Docking:

In order to explore the binding mode and understanding of key active site residues, molecular docking study has been performed. The synthesized quinazolinone series of compounds were docked into the active site of HDAC8 and EHMT2 proteins. In the present study, the docking result of compound (**5c**) in HDAC8 and EHMT2 proteins are discussed.

HDAC8: From the docking study, we observed that the non-covalent hydrophobic interactions as well as electrostatic interactions are more predominating for strong binding towards HDAC8 protein. The oxygen of 2,3-dihydro-1*H*-quinazolinone moiety formed salt bridge with aspartic acid (Asp178) and histidine residue (His180) through Zn^{+2}. Also the same oxygen atom showed hydrogen bond interaction with methionine (Met274, 3.40Å). The nitrogen atom of 2,3-dihydro-1*H*-quinazolinone showed hydrogen bond interaction with carbonyl oxygen of glycine (Gly151, 3.16 Å) backbone residue. The phenyl ring of 2,3-dihydro-1*H*-quinazolinone moiety and sulfonyl piperazine moieties showed strong noncovalent hydrophobic interactions with two phenylalanine residues (Phe152, Phe208) and methionine (Met274) residue. 2,4-dichloro-phenyl moiety is oriented towards hydrophobic

pocket containing tyrosine (Tyr306) and tryptophan (Trp141) residues.

EHMT2: From the docking results, we observed that the non-covalent hydrophobic interactions are more predominating as compared to electrostatic interactions for binding of the molecules. The active site comprises mostly the hydrophobic residues include tyrosine (Tyr1085, Tyr1067), phenyl alanine (Phe1110, Phe1138, Phe1158, Phe1087) and proline (Pro1121, Pro1011) and isoleucine (Ile1136, Ile1009, Ile1120). The nitrogen atom of 2,3-dihydro-1*H*-quinazolinone showed hydrogen bond interaction with aspartic acid (Asp1088, 3.16 Å). The fluoro atom of fluorophenyl moiety showed electrostatic interaction with positively charged arginine residue (Arg1157, 3.10 Å). The oxygen atom of 2,3-dihydro-1*H*-quinazolinone moiety oriented towards positively charged lysine residue (Lys1162). Hydrophobic 2,4-dichloro-phenyl moiety showed strong non-covalent hydrophobic interactions with phenyl alanine residues (Phe1087, Phe1152, Phe1158) and tyrosine residue (Tyr1154) in the active site of EHMT2. The probable binding mode of most potent compound (**5c**) of the quinazolinone series of compounds in EHMT2 and HDAC8 is shown in Figure 3. The active site residues around 4Å of the most potent compound (**5c**) of the series are shown in Table 1.

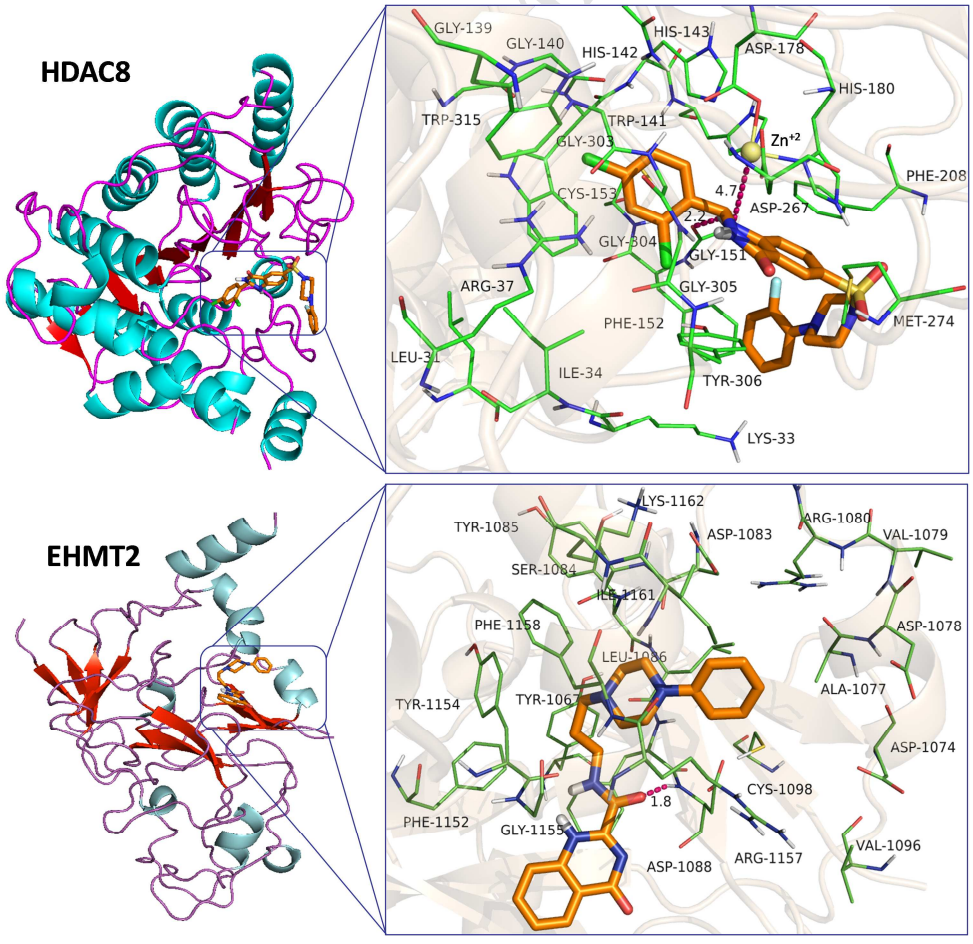


Figure 3. The probable binding mode compound (**5c**, orange) in the active site of HDAC8 and EHMT2 proteins (helix: cyan, sheet: red and loop: purple). The hydrogen bond interactions are shown in hot pink colored dotted lines, metal Zn^{+2} is shown in yellow color, the important residues are shown in green color.

Target	**Compound 10c (Active site residues around 4Å)**
HDAC8	ARG37, GLY139, GLY140, TRP141, HIS142, HIS143, GLY151, PHE152, CYS153, HIS180, PHE208, GLN263, ASP267, MET274, GLY303, GLY304, GLY305, TYR306, ARG37, GLY139, GLY140, TRP141, HIS142, HIS143, GLY151, PHE152, CYS153, HIS180, PHE208, GLN263, ASP267, MET274, GLY303, GLY304, GLY305, TYR306
EHMT2	ASP1074, ALA1077, ASP1078, ASP1083, SER1084, LEU1086, PHE1087, ASP1088, PRO1121, PHE1152, ASP1153, TYR1154, ARG1157, PHE1158, ILE1161, LYS1162

Table 1: The active site residues around 4Å of the most potent compound (**5c**)

Table-2: ($GI_{50}/\mu M)^a$ values of the tested compounds against four human cancer cell lines.

Sr. No.	**Compounds**	**HeLa**	**MDA-MB-231**	**PANC-1**	**A549**
1	**5a**	0.675 ± 0.02	6.325 ± 0.01	2.955 ± 0.01	3.455 ± 0.02
2	**5b**	1.286 ± 0.01	0.324 ± 0.02	2.362 ± 0.02	2.2 ± 0.01
3	**5c**	0.124 ± 0.02	0.095 ± 0.001	0.212 ± 0.02	0.115 ± 0.01
4	**5d**	2.2 ± 0.01	1.162 ± 0.02	3.697 ± 0.01	1.946 ± 0.02
5	**5e**	3.344 ± 0.03	6.94 ± 0.02	6.854 ± 0.01	4.324 ± 0.02
6	**5f**	1.333 ± 0.02	0.356 ± 0.01	1.412 ± 0.01	1.772 ± 0.01
7	**5g**	2.534 ± 0.01	0.297 ± 0.03	1.778 ± 0.02	2.358 ± 0.02
8	**5h**	1.858 ± 0.01	2.371 ± 0.01	2.894 ± 0.02	1.864 ± 0.02
9	**5i**	0.045 ± 0.003	0.194 ± 0.02	6.737 ± 0.03	0.358 ± 0.01
10	**5j**	1.661 ± 0.02	1.296 ± 0.02	7.257 ± 0.02	4.736 ± 0.02
11	**6a**	2.863 ± 0.01	5.963 ± 0.01	6.128 ± 0.01	2.128 ± 0.03

12	**6b**	1.683 ± 0.02	8.317 ± 0.03	6.422 ± 0.02	2.783 ± 0.03
13	**6c**	0.316 ± 0.01	1.743 ± 0.01	1.582 ± 0.02	3.783 ± 0.01
14	**6d**	2.414 ± 0.02	4.283 ± 0.01	3.452 ± 0.02	4.572 ± 0.01
15	**6e**	0.446 ± 0.02	4.337 ± 0.02	0.483 ± 0.02	3.542 ± 0.03
16	**6f**	0.296 ± 0.01	0.795 ± 0.01	1.743 ± 0.02	0.644 ± 0.02
17	**6g**	4.521 ± 0.02	0.617 ± 0.02	1.895 ± 0.02	2.764 ± 0.02
18	**Paclitaxel[b]**	**0.02 ± 0.002**	**0.03 ± 0.001**	**< 0.01 ± 0.003**	**0.02 ± 0.002**
19	**Nocadazole[b]**	**<0.01 ± 0.001**	**<0.01 ± 0.001**	**< 0.01 ± 0.003**	**0.03 ± 0.001**
20	**Colchicine[b]**	**<0.01 ± 0.002**	**0.02 ± 0.003**	**0.02 ± 0.002**	**<0.01 ± 0.002**
21	**Cambretostatin (CA4)[b]**	**0.02 ± 0.002**	**<0.01 ± 0.001**	**<0.01 ± 0.002**	**0.01 ± 0.002**
22	**Doxorubicin[b]**	**0.03 ± 0.003**	**0.01 ± 0.002**	**< 0.01 ± 0.003**	**<0.01 ± 0.003**

[a]GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net rotein increase compared with control cells. [b]Positive control.

Conclusion

In conclusion, a novel sulphamide tethered quinazolinones hybrids as antitumor agents have been synthesized. Compounds **5a-j, 6a-g** were synthesized and screened for viability assays against human HeLa (cervical), MDA-MB-231 (breast), PANC-1 (pancreatic), and A549 (lung carcinoma) cell lines *in vitro*. In general, most of the prepared compounds showed significant cytotoxic effect against human tumor cell lines. Compounds **5c** showed potent cytotoxic activity against all the four human cancer cell lines with GI_{50} values ranging from 0.09 to 0.21 μM. Also compounds **5i** and **6f** showed potent anti-cytotoxic activity against the three human cancer cell lines HeLa, MDA-MB-231, and A549 with GI_{50} values ranging from 0.045 to 0.35 μM. We succeeded in the substituent change on sulphamide and propyl linker which played a critical role in exhibiting promising anti-proliferative activities. This study provides that these compounds would be very useful for further investigation of tumor cell metastasis, ant-proliferative mechanism and angiogenesis in our next research project and one of them could be potential antitumor agent. We have identified five featured pharmacophore (ADHRR) and from the docking study, we have explored the probable binding mode and identified key active site residues around potent compound (**5c**) in HDAC8 and EHMT2 proteins.

Experimental

General chemistry:

All the chemicals and reagents were purchased from Sigma-Aldrich and SD Fine-Chemicals, Pvt. Ltd. India, and used as received. The reactions were monitored and R_f value was determined using analytical thin layer chromatography (TLC) with Merck Silica gel 60-120 and F_{254} pre-coated plates (0.25 mm thickness). Spot on the TLC plates were visualized using ultraviolet light (254 nm). Flash column chromatography was performed with Merck silica gel (100-200 mesh). Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker DRX-300, Avance-300and Avance new-500 NMR spectrometers. ^{13}C NMR spectra were recorded on Bruker DRX-300. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.00 or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, δ 7.26; DMSO-d_6 δ 2.54) and multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines). Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 400 spectrometer; data is reported in wave numbers (cm^{-1}). Mass spectra were recorded on Agilent Technologies 1100 Series (Agilent Chemistation Software). High-resolution mass spectra (HRMS) were obtained by using ESI-QTOF mass spectrometry.

Preparation of 2-(2,4-dichlorophenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (4).

A solution of 2-aminobenzamide (3g, 0.02 mol), 2, 4-dichloro benzaldehyde (3.7g, 0.02 mol) and a catalytic amount of TBAHS (1 mol%) in MeOH was made to react at reflux temperature for 2 h. The reaction mixture was then cooled to rt and small amount of water (15 mL) was added to the mixture, and then generated solid was filtered to give cyclic compound **3**. The intermediate **3** converted into **4** by the drop wise addition of Chloro sulphonic acid (15 mL) and stirred for 25 min at -10°C. The reaction mixture was carefully poured into ice. The cold water was washed with CH_2Cl_2. CH_2Cl_2 was separated, dried over Na_2SO_4 and was concentrated by evaporation under vacuum to furnish a light yellow solid.

Synthesis of 2-(3-bromopropyl) isoindoline-1, 3-dione (7).

To a stirred solution of isoindoline-1, 3-dione (10g, 1mmol) in DMF (40 mL), 1,3-dibromo propane (14g, 1 mmol), K_2CO_3 (9.3 g, 3 mmol) were added at rt. The reaction

mixture was stirred at rt for 3 h. After completion of the reaction, as indicated by TLC, The resulting solution was poured into ice cold water, which was then extracted with ethyl acetate. Ethyl acetate was separated, dried over Na_2SO_4 and concentrated under vacuum to afford a white solid. Yield 85%; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.23-2.33 (m, 2H, CH_2), 3.42 (t, *J* = 6.4 Hz, 2H, CH_2), 3.86 (t, *J* = 6.4, 6.9 Hz, 2H, CH_2), 7.74 (m, 2H, Ar-H), 7.86 (m, 2H, Ar-H); ESI MS : *m/z* = 268 $[M+H]^+$.

General procedure for the synthesis of compounds 5a-j, 6a-g.

2-(2,4-dichlorophenyl)-4-oxo-1,2,3,4-tetrahydroquina zoline-6-sulfonyl chloride (0.1g, 0.0002 mol) was added to a round bottom flask containing *N*,*N*-dimethylformamide (4 mL) under nitrogen atmosphere, *N*-phenyl piperzine derivatives (0.0002 mol) or 3-(4-phenylpiperazin-1-yl)propan-1-amine derivatives (0.0002 mol) was added at rt with stirring, followed by the addition of DIEA (1 mL). The stirring was continued for 1 h. After completion of the reaction, as indicated by TLC, The ice cold water was added to the reaction mixture stirred it for 5 min and then extract with Chloroform (10 mL). The chloroform layer was separated, dried over Na_2SO_4 and evaporated under vacuum to give corresponding sulphamide derivatives (**5a-j**, **6a-g**) in good yields.

2-(2,4-Dichlorophenyl)-6-((4-(4-nitrophenyl)piperazin-1-yl)sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5a). Yield 85%; mp 138-141 ^{o}C; IR (KBr) υ in cm^{-1}: 1134, 1239, 1372, 1634, 3607; ^{1}H NMR(300 MHz, $CDCl_3$): δ 2.96 (s, 4H, 2xCH_2), 3.52 (s, 4H, 2xCH_2), 6.33 (s, 1H, CH), 6.78-6.90 (m, 3H, Ar-H), 7.30 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.57 (s, 2H, Ar-H), 7.62 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.92 (bs, 1H, NH), 8.07 (d, *J* = 9.0 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 44.3, 45.3, 62.3, 112.2, 113.9, 121.1, 124.4, 126.3, 127.3, 128.1, 128.5, 131.4, 131.8, 133.7, 135.2, 137.1, 149.5, 152.9, 161.3; ESI MS : *m/z* = 562 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-6-((4-(2-methoxyphenyl)piperazin-1-yl)sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5b). Yield 81%; mp 147-150 ^{o}C; IR (KBr) υ in cm^{-1}: 1162, 1243, 1344, 1662, 3360; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75-2.02 (bs, 8H, 4xCH_2), 2.59 (s, 3H, OCH_3), 5.11 (s, 1H, CH), 5.56-5.71 (bs, 3H, Ar-H), 5.72-5.82 (m, 1H, Ar-H), 6.06-6.16 (m, 1H, Ar-H), 6.22 (s, 1H, Ar-H), 6.34 (s, 1H, Ar-H), 6.43 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 6.94 (d, *J* = 11.5 Hz, 1H, NH), 7.41 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 44.9, 48.3, 54.0, 62.2, 109.9, 112.1, 113.4, 116.9, 121.3, 122.0, 126.3, 127.3, 127.9, 128.4, 131.4, 131.7, 133.6,

135.2, 138.9, 149.3, 150.6, 161.4; ESI MS : *m/z* = 547 $[M+H]^+$; HRMS calculated for $C_{25}H_{25}Cl_2N_4O_4S$: 547.0900, Found : 547.0974.

2-(2,4-Dichlorophenyl)-6-((4-(2-fluorophenyl)piperazin-1-yl)sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5c). Yield 87%; mp 142-145 ^oC; IR (KBr) υ in cm^{-1}: 1134, 1237, 1370, 1669, 3653; ^1H NMR(300 MHz, $CDCl_3$): δ 3.05 (t, *J* = 5.1, 10.2 Hz, 2H, CH_2), 3.10 (t, *J* = 5.0, 10.0 Hz, 2H, CH_2), 3.55 (t, *J* = 4.8, 10.0 Hz, 2H, CH_2), 3.74 (t, *J* = 4.8, 10.2 Hz, 2H, CH_2), 6.91-6.96 (m, 1H, Ar-H), 6.97-7.02 (m, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 7.04-7.10 (m, 2H, Ar-H), 7.29-7.38 (m, 1H, Ar-H), 7.40-7.48 (m, 1H, Ar-H), 7.50-7.56 (m, 1H, Ar-H), 7.61-7.74 (m, 1H, Ar-H), 8.04 (dd, *J* = 7.7, 9.1 Hz, 1H, Ar-H), 8.10 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 40.1, 45.7, 50.1, 116.2, 116.3, 119.3, 123.3, 123.4, 124.5, 126.8, 128.8, 130.9, 133.3, 134.0, 137.0, 139.5, 144.4, 147.8, 151.0, 154.6, 156.7, 160.8; ESI MS : *m/z* = 532 $[M-2H]^+$.

2-(2,4-Dichlorophenyl)-6-((4-(4-fluorophenyl)piperazin-1-yl)sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5d). Yield 89%; mp 136-139 ^oC; IR (KBr) υ in cm^{-1}: 1191, 1293, 1315, 1616, 3661; ^1H NMR(300 MHz, $CDCl_3$): δ 1.25 (bs, 4H, 2xCH_2), 1.81 (bs, 4H, 2xCH_2), 5.46 (s, 1H, CH), 7.33-7.43 (m, 2H, Ar-H), 7.50-7.61 (m, 2H, Ar-H), 7.74 (t, *J* = 7.3, 14.1 Hz, 1H, Ar-H), 7.89 (t, *J* = 7.5, 15.1 Hz, 1H, Ar-H), 7.98 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.61 (d, *J* = 4.1 Hz, 1H, Ar-H), 8.81 (d, *J* = 4.5 Hz, 1H, Ar-H), 10.09 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 51.6, 52.7, 58.6, 107.7, 109.0, 110.5, 111.9, 114.4, 120.4, 123.2, 126.7, 128.8, 129.5, 131.6, 134.0, 135.8, 137.4, 140.9, 142.6, 148.6, 153.2, 160.3; ESI MS : *m/z* = 535 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-6-((4-methylpiperazin-1-yl) sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5e). Yield 82%; mp 137-140 ^oC; IR (KBr) υ in cm^{-1}: 1239, 1372, 1740, 2920; ^1H NMR(300 MHz, $CDCl_3$): δ 2.06 (s, 3H, CH_3), 2.18 (t, *J* = 7.3, 14.6 Hz, 4H, 2xCH_2), 3.01 (t, *J* = 5.1, 10.6 Hz, 4H, 2xCH_2), 5.73 (s, 1H, CH), 7.16-7.91 (m, 5H, Ar-H), 8.30 (bs, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 28.1, 44.5, 45.6, 58.6, 111.5, 113.8, 116.7, 124.9, 126.3, 127.8, 130.6, 131.5, 135.1, 150.9, 164.9, 168.9; ESI MS : *m/z* = 455 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-6-((4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5f). Yield 89%; mp 142-145 ^oC; IR (KBr) υ in cm^{-1}: 1134, 1293, 1323, 1667, 3672; ^1H NMR(300 MHz, $CDCl_3$): δ 2.50 (t, *J* = 1.8, 3.6 Hz, 2H, CH_2), 2.99 (t, *J*

= 4.7, 9.6 Hz, 2H, CH_2), 3.38 (bs, 4H, 2xCH_2), 6.31 (s, 1H, CH), 6.90 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.04 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.50 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.6, 10.9 Hz, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.70 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.97 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.01 (bs, 1H, Ar-H), 8.56 (bs, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 51.6, 52.4, 62.3, 112.2, 114.5, 124.4, 126.3, 128.1, 128.5, 131.3, 133.7, 135.2, 137.2, 141.1, 143.1, 149.1, 152.9, 161.2; ESI MS : *m/z* = 585 $[M+H]^+$; HRMS calculated for $C_{25}H_{22}Cl_2F_3N_4O_3S$: 585.0700, Found : 585.0749.

tert-Butyl4-((2-(2,4-dichlorophenyl)-4-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)sulfonyl)piperazine-1-carboxylate (5g). Yield 85%; mp 139-142 ^oC; IR (KBr) υ in cm^{-1}: 1173, 1321, 1662, 3284; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.41 (s, 9H, 3xCH_3), 2.92 (bs, 4H, 2xCH_2), 3.48 (bs, 4H, 2xCH_2), 6.35 (s, 1H, CH), 6.89 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.53 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.59 (s, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 8.12 (s, 1H, NH), 8.21 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 26.5, 44.1, 61.8, 78.0, 111.6, 113.3, 120.8, 126.0, 126.7, 127.5, 128.2, 130.9, 131.4, 133.1, 134.9, 149.1, 152.1, 160.8; ESI MS : *m/z* = 541 $[M+H]^+$.

6-((4-Benzylpiperidin-1-yl)sulfonyl)-2-(2,4-dichlorophenyl)-2,3-dihydroquinazolin-4(1H)-one (5h). Yield 85%; mp 142-145 ^oC; IR (KBr) υ in cm^{-1}: 1161, 1336, 1655, 3367; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.66 (d, *J* = 12.4 Hz, 2H, CH_2), 2.19 (t, *J* = 10.5, 11.7 Hz, 2H, CH_2), 2.50 (d, *J* = 6.8 Hz, 2H, Ph-CH_2), 2.58 (bs, 1H, CH), 3.16 (bs, 4H, 2xCH_2), 6.33 (s, 1H, CH), 6.85 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.08 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.16 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.24 (t, *J* = 7.5, 14.7 Hz, 2H, Ar-H), 7.35 (dd, *J* = 8.4, 10.1 Hz, 1H, Ar-H), 7.45 (bs, 1H, Ar-H), 7.49 (bs, 1H, Ar-H), 7.65 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 8.09 (s, 1H, NH), 8.15 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 29.4, 35.2, 40.6, 44.7, 61.9, 111.6, 113.1, 121.8, 124.3, 126.1, 126.5, 127.3, 127.6, 128.3, 130.9, 131.5, 133.1, 135.0, 138.1, 148.8; ESI MS : *m/z* = 530 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-6-(thiomorpholinosulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5i). Yield 82%; mp 148-151 ^oC; IR (KBr) υ in cm^{-1}: 1134, 1317, 1635, 3478; ^{1}H NMR(300 MHz, $CDCl_3$): δ 2.74 (bs, 4H, 2xCH_2), 3.31 (bs, 4H, 2xCH_2), 6.37 (d, *J* = 6.0 Hz, 1H, CH), 6.87 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.34 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.46 (d, *J* = 11.1 Hz, 1H, Ar-H), 7.54-7.62 (m, 1H, Ar-H), 7.65 (bs, 1H, NH), 8.22 (d, *J* = 12.8 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 28.0, 46.3, 58.5, 111.5, 113.7, 116.7, 123.1, 124.9, 126.3, 127.8, 130.6, 131.5, 135.1, 140.3, 164.8, 168.9; ESI MS : *m/z* = 458 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-6-((4-phenylpiperazin-1-yl)sulfonyl)-2,3-dihydroquinazolin-4(1H)-one (5j). Yield 81%; mp 147-150 ^{o}C; IR (KBr) υ in cm^{-1}: 1134, 1292, 1540, 1794, 3630; ^{1}H NMR(300 MHz, $CDCl_3$): δ 2.37 (bs, 4H, 2xCH_2), 2.64 (bs, 4H, 2xCH_2), 5.07 (s, 1H, CH), 6.11 (d, *J* = 15.2 Hz, 1H, Ar-H), 6.63 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.92 (d, *J* = 9.7 Hz, 2H, Ar-H), 7.09 (s, 1H, Ar-H), 7.12-7.18 (m, 2H, Ar-H), 7.31 (t, *J* = 8.0, 16.0 Hz, 1H, Ar-H), 7.36-7.46 (m, 2H, Ar-H), 7.95 (d, *J* = 8.8 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 55.5, 56.1, 62.3, 112.2, 114.0, 121.1, 124.5, 126.3, 127.3, 128.1, 128.6, 131.4, 131.8, 133.8, 135.2, 137.1, 143.1, 149.6, 153.0, 161.4; ESI MS : *m/z* = 517 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-N-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6a). Yield 79%; mp 141-144 ^{o}C; IR (KBr) υ in cm^{-1}: 1140, 1241, 1384, 1665, 3673; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.63-1.82 (m, 2H, CH_2), 2.60 (t, *J* = 6.0, 11.7 Hz, 2H, CH_2), 2.75 (bs, 4H, 2xCH_2), 3.03 (t, *J* = 6.0, 11.1 Hz, 2H, CH_2), 3.12 (bs, 4H, 2xCH_2), 3.84 (s, 3H, OCH_3), 5.54 (bs, 1H, NH), 6.33 (s, 1H, CH), 6.67-7.06 (m, 6H, Ar-H), 7.24 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.39 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.55 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.73 (dd, *J* = 6.6, 8.5 Hz, 1H, SO_2NH), 8.00 (bs, 1H, NH), 8.33 (d, *J* = 1.8 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 29.0, 49.9, 52.7, 54.8, 56.6, 63.3, 110.8, 113.1, 114.2, 117.7, 120.4, 122.3, 127.1, 127.4, 128.5, 128.9, 131.7, 132.4, 134.8, 135.7, 140.5, 141.3, 149.5, 151.7, 162.5; ESI MS : *m/z* = 604 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-N-(3-(4-(2-fluorophenyl)piperazin-1-yl)propyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6b). Yield 78%; mp 151-154 ^{o}C; IR (KBr) υ in cm^{-1}: 1154, 1238, 1384, 1651, 3451; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.71 (bs, 2H, CH_2), 2.54 (bs, 2H, CH_2), 2.65 (bs, 4H, 2xCH_2), 3.03 (bs, 2H, CH_2), 3.14 (bs, 4H, 2xCH_2), 6.35 (s, 1H, CH), 6.74 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.88 (bs, 3H, Ar-H), 6.91-6.99 (m, 3H, Ar-H), 7.40 (d, *J* = 16.0 Hz, 1H, Ar-H), 7.56 (t, *J* = 8.3, 16.7 Hz, 1H, Ar-H), 7.72 (br, 1H, SO_2NH), 7.99 (br, 1H, NH), 8.31 (d, *J* = 10.0 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 50.0, 53.2, 54.8, 56.1, 63.3, 110.9, 113.1, 114.2, 117.7, 120.5, 122.3, 127.1, 127.4, 128.5, 129.0, 131.7, 132.5, 134.9, 135.2, 140.5, 149.5, 151.7, 162.4; ESI MS : *m/z* = 592 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-4-oxo-N-(3-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)propyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6c). Yield 79%; mp 145-148 ^{o}C; IR (KBr) υ in cm^{-1}: 1134, 1323, 1667, 2965, 3672; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.62-1.75 (m, 2H, CH_2),

2.49 (bs, 2H, CH_2), 2.59 (bs, 4H, 2xCH_2), 3.03 (bs, 2H, CH_2), 3.28 (bs, 4H, 2xCH_2), 5.50 (br, 1H, NH), 6.33 (s, 1H, CH), 6.73 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.89-6.98 (m, 3H, Ar-H), 7.39 (bs, 1H, Ar-H), 7.46 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.55 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.72 (dd, *J* = 7.1, 8.4 Hz, 1H, SO_2NH), 7.98 (br, 1H, NH), 8.31 (br, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 43.5, 47.8, 52.7, 57.4, 63.9, 114.0, 114.6, 114.7, 126.3, 126.3, 126.4, 128.0, 128.7, 128.8, 129.9, 130.3, 130.8, 132.8, 132.9, 135.0, 136.1, 148.9, 153.0, 163.1; ESI MS : *m/z* = 642 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-4-oxo-N-(3-(4-phenylpiperazin-1-yl)propyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6d). Yield 82%; mp 152-155 ^oC; IR (KBr) υ in cm^{-1}: 1157, 1319, 1667, 2926, 3640; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.55-1.75 (m, 2H, CH_2), 2.45 (t, *J* = 6.2, 12.6 Hz, 2H, CH_2), 2.58 (bs, 4H, 2xCH_2), 2.94 (t, *J* = 6.4, 15.8 Hz, 2H, CH_2), 3.18 (bs, 4H, 2xCH_2), 6.33 (s, 1H, CH), 6.79-6.95 (m, 3H, Ar-H), 7.19-7.36 (m, 3H, Ar-H), 7.44 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.60-7.69 (m, 3H, Ar-H), 7.86 (bs, 1H, SO_2NH), 8.00 (bs, 1H, NH), 8.28 (d, *J* = 2.0 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 43.5, 48.9, 52.9, 57.3, 63.8, 113.8, 114.7, 116.1, 119.9, 128.0, 128.1, 128.7, 128.8, 129.0, 129.9, 130.0, 130.8, 132.8, 135.1, 136.0, 149.0, 150.8, 163.2; ESI MS : *m/z* = 574 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-4-oxo-N-(3-thiomorpholinopropyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6e). Yield 81%; mp 151-154 ^oC; IR (KBr) υ in cm^{-1}: 1005, 1318, 1667, 3526; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.58-1.70 (m, 2H, CH_2), 2.44 (t, *J* = 6.0, 12.0 Hz, 2H, CH_2), 2.69 (s, 8H, 4xCH_2), 3.00 (t, *J* = 5.8, 11.7 Hz, 2H, CH_2), 5.54 (s, 1H, NH), 6.36 (s, 1H, CH), 6.75 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 7.29 (dd, *J* = 6.4, 8.5 Hz, 1H, Ar-H), 7.43 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.59 (d, *J* = 8.3 Hz, 1H, SO_2NH), 7.72 (dd, *J* = 6.4, 8.5 Hz, 1H, Ar-H), 7.99 (bs, 1H, NH), 8.29 (d, *J* = 1.8 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 26.6, 27.1, 45.9, 54.3, 56.7, 63.3, 113.1, 114.2, 121.2, 127.4, 128.5, 129.0, 131.9, 132.4, 134.9, 135.7, 137.3, 149.5, 162.5; ESI MS : *m/z* = 515 $[M+H]^+$; HRMS calculated for $C_{21}H_{25}Cl_2N_4O_3S_2$: 515.0700, Found : 515.073.

N-(3-(4-Benzylpiperidin-1-yl)propyl)-2-(2,4-dichlorophenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6f). Yield 78%; mp 149-153 ^oC; IR (KBr) υ in cm^{-1}: 1156, 1321, 1666, 3239; ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.31-1.48 (m, 2H, CH_2), 1.49-1.79 (m, 5H, 2xCH_2, CH), 2.10 (t, *J* = 10.3, 11.7 Hz, 2H, CH_2), 2.52 (d, *J* = 6.6 Hz, 2H, $PhCH_2$), 2.61 (bs, 4H, 2xCH_2), 3.08 (d, *J* = 10.3 Hz, 2H, CH_2), 5.74 (bs, 1H, NH), 6.33 (s, 1H, CH), 6.76 (d, *J* = 8.5 Hz, 1H,

Ar-H), 6.90 (bs, 1H, Ar-H), 7.12 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.19 (d, *J* = 6.6 Hz, 1H, Ar-H), 7.22-7.31 (m, 3H, Ar-H), 7.38 (s, 1H, Ar-H), 7.55 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.70 (d, *J* = 8.5 Hz, 1H, SO_2NH), 8.14 (d, *J* = 15.9 Hz, 1H, NH), 8.30 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 24.0, 29.7, 30.8, 37.0, 42.4, 53.4, 56.7, 63.9, 113.6, 115.0, 125.9, 127.9, 128.1, 128.2, 129.0, 129.6, 129.8, 132.7, 132.7, 135.3, 135.8, 139.9, 149.2, 163.1; ESI MS : *m/z* = 587 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-N-(3-(4-(4-nitrophenyl)piperazin-1-yl)propyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6g). Yield 79%; mp 148-151 ^oC; IR (KBr) υ in cm^{-1}: 1050, 1378, 1470, 1681, 3181; ^1H NMR(300 MHz, $CDCl_3$): δ 0.77-0.95 (m, 2H, CH_2), 2.07 (s, 2H, CH_2), 2.59 (bs, 2H, CH_2), 3.17 (bs, 8H, 4xCH_2), 5.90 (bs, 1H, NH), 6.24 (d, *J* = 10.9 Hz, 1H, Ar-H), 6.50 (s, 1H, CH), 6.76 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.23-7.32 (m, 1H, Ar-H), 7.34-7.45 (m, 2H, Ar-H), 7.49-7.81 (m, 3H, Ar-H), 7.92 (d, *J* = 8.4 Hz, 1H, SO_2NH), 8.08 (d, *J* = 8.6 Hz, 1H, Ar-H), 8.17-8.33 (m, 1H, Ar-H), 8.50 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 43.6, 47.7, 52.8, 57.7, 64.0, 114.0, 114.7, 126.3, 126.4, 126.4, 128.1, 128.7, 128.8, 140.1, 142.7, 143.1, 149.2, 154.1, 163.1; ESI MS : *m/z* = 616 $[M-2H]^+$.

Materials and Methods:

Cell Cultures, Maintenance and Anti proliferative Evaluation

The cell lines, HeLa (cervical), MDA-MB-231 (breast), PANC-1 (pancreatic), and A549 (lung carcinoma) which were used in this study were procured from American Type Culture Collection (ATCC), United States. The synthesized test compounds were evaluated for their *in vitro* anti proliferative activity in these four different human cancer cell lines. A protocol of 48 h continuous drug exposure was used and an SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 μL aliquots at plating densities depending on the doubling time of individual cell lines. The micro titre plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, 100μM) of prepared derivatives**.** After 48 hours incubation at 37 °C, cell mono layers were fixed by the addition of 10% (wt/ vol) cold trichloroacetic acid and incubated at 4 °C for 1 h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein –bound dye was dissolved in 10 mM Tris base solution for OD

determination at 510 nm using a micro plate reader (Enspire, Perkin Elmer, and USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

The dose response parameter, growth inhibition of 50 % (GI50) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for this parameter if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

Methadology for modelling:

Dataset preparation

A dataset comprising of 17 quinazolin-4-one series of compounds and these compounds were drawn with all possible tautomers, protonated states and were minimized with OPLS-2005 force field using water as solvent in the GB/SA continuum solvation model [40]. The minimization of molecules was carried out using Polak-Ribiere Conjugate Gradient (PRCG) method with maximum of 5000 iterations. Further for the generation of pharmacophore model, the minimized structures were subjected to extensive conformational search with Mixed torsional/Low-mode sampling method with the use of 100 steps per rotatable bond, maximum number of steps 1000, energy window for saving structures with 5.02 kcal/mol, eliminate the redundant conformers with maximum atom deviation cut-off 0.5 Å and saved 250 structures for each search. The generated conformers were used for the generation of pharmacophore modelling and docking studies.

Pharmacophore modeling:

The pharmacophore model was generated using Phase module of Schrödinger software v9.3. The set of pharmacophore features, such as hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively ionizable (N), positively ionizable (P), and aromatic ring (R), were created for a series of compounds containing A549 cell-line activity.

Protein preparation:

These series of compounds have showed strong affinity, intrinsic activity towards various cancer cell-lines and showed inhibition against Histone deacetylase 8 (HDAC 8) and Histone-lysine N-methyltransferase, H3 lysine-9 specific 3 (EHMT2). Hence in our current study, we have carried out molecular docking study have been performed to understand the key active site residues HDAC8, EHMT2 proteins and explored all probable binding modes. The crystal structure of human HDAC 8 (PDB ID: 3SFH) and human EHMT2 (PDB ID: 3K5K) have been selected for the docking studies. The 3SFH and 3K5K structures were prepared by adjusting bond orders, tautomers and adding hydrogen atoms using protein preparation wizard of Schrödinger software graphical user interface Maestro v9.3 [Maestro, version 9.3, Glide version 2.8, Schrödinger, LLC, New York, NY 2013.]. Further the protein was minimized by OPLS-2005 force field with converge heavy atoms to RMSD 0.3 Å relative to original protein structure.

Docking studies:

The dataset compounds were docked into the prepared 3SFH and 3K5K crystal structures using Glide module of Schrödinger suite [41]. The prepared protein structures were used for grid generation using the default value of protein atom scaling (1.0) within a cubic box centered on the co-crystal ligand. No constraints were imposed and extra precision (XP) flexible docking of ligands was carried out with default value of ligand atom scaling (0.8). The post docking minimization has been carried out and maximum of 10 poses per ligand was saved. The obtained docked complex structures were analyzed and the compounds were prioritized by using docking score, interactions with active site residues.

Acknowledgments

The authors gratefully acknowledge the financial support through the project: **DST-SERB/EEQ/2017/095** RV thanks CSIR, New Delhi for award of research fellowship.

Supplementary data

Experimental section and Copies of the ^{1}H, ^{13}C NMR, ESI-MS and HRMS spectra for some of the important compounds.

References

1. G. Bonola, P. Da Re, M.J. Magistretti, E. Massarani, and I. Setnikar, *J. Med. Chem*, **1968,** *11*, 1136.
2. K. Okumura, T. Oine, Y. Yamada, G. Hayashi, and M. Nakama, *J. Med. Chem*, **1968,** *11*, 348.
3. J.H. Chan, J.S. Hong, L.F. Kuyper, M.L. Jones, D.P. Baccanari, R.L. Tansik, C.M. Boytos, S.K. Rudolph, and A.D. Brown, *J. Heterocycl. Chem*, **1997,** *34*, 145.
4. S.L. Gackenheimer, J.M. Schaus, and D.R. Gehlert, J. Pharmacol. Exp. Ther. **1996,** *113***,** 732.
5. R.O. Dempcy and E.B. Skibo, Biochemistry **1991,** ***30***, 8480.
6. P.Y. Shirodkar and M. VartakMeghna, *Indian J. Heterocyclic Che,* **2000,** *9***,** 239.
7. V.K. Pandey, D. Misra, and A. Shukla, Indian Drugs **1994,** *31*, 532.
8. N.C. Desai, B.R. Shah, J.J. Bhatt, H.H. Patel, N.K. Undavia, P.B. Trivedi, and V. Narayanan, *Indian J. Chem*, **1995,** *34B***,** 201.
9. L.M. Werbel, E.F. Elslager, and L.S. Newton, *J. Heterocyclic Che,* **1987,** *24*, 252.
10. N.B. Patel and J.D. Lilakar, *Indian J. Heterocyclic Chem*, **2001,** *11*, 85.
11. a) K.K. Naveen, S. Dey, M. Sajid, M. Owais , N. Ahmed, *Eur. J. Med. Chem,* **2013**, *59*, 23 b) N. Ahmed, K.K. Naveen, S. Ahmad, M. Owais, *Eur. J. Med. Chem,* **2014**, *82*, 552.
12. H. Zafar, A. Kareem, A. Sherwani, M. Owais, T.A. Khan, *Jour. Mol. Str.* **2014**, *1079*, 337.
13. A. Kareem, H. Zafar, A. Sherwani, M. Owais, T.A. Khan. *Jour. Mol. Str*, **2015**, *1075*, 17.
14. H. Zafar, A. Kareem, A. Sherwani, M. Owais, M. A. Ansari, H.M. Khan, T.A. Khan, *Jour. Mol. Str.* **2014**, *1085*, 104.
15. M. Shakir, S. Hanif, M.A. Sherwani, M. Owais, I. Saud, Al-Resayes. *Jour. Mol. Str.* **2015,** *1092* 143.
16. H. Zafar, A. Kareem, A. Sherwani, M. Owais, M.A. Ansari, H.M. Khan, T.A. Khan, *Jour. Phot. chem.Phot.bio.* **2015**, *142*, 8.
17. Z.Z. Ma, Y. Hano, T. Nomura, and Y.J. Chen, *Heterocycles,* **1997,** *46*, 541.
18. M.E. Wall, M.C. Qani, C.E. Cook, K.H. Palmer, A.T. Mcphail, and G.A. Sim, *J. Am. Chem. Soc*. **1966,** *88*, 3888.
19. C.H. Takimoto, J. Wright, and S.G. Arbuck. Biochim. Biophys. Acta **1998,** ***1400***, 107.

20. J. O'Leary and F.M. Muggia, *Eur. J. Cancer* **1998,** *34*, 1500.
21. L.B. Saltz, J.V. Cox, C. Blanke, L.S. Rosen, L. Fechrenbacher, M.J. Moore, J.A. Maroun, S.P. Ackland, P.K. Locker, N. Pirotta, G.L. Elfring, and L.L. Miller. New Engl, *J. Med*, **2000,** *343*, 905.
22. R.F. Ozols. Int. J. Gynecol. Cancer **2000,** *10*, 33.
23. A. VanhoHarstrick, W. Achterrath, S. Cao, S. Seeber, and Y.M. Rustum, *J. Clin. Oncol***. 2001,** *19*, 1501.
24. H. Ulukan, and P.W. Swaan, Drugs **2002**, *62*, 2039.
25. R. Garcia-Carbenero, and J.G. Supko. Clin. Cancer. Res**. 2002,** *8*, 641.
26. For example, Raltitrexed (Tomudex, marketed for colorectal cancer), Ispinesib (phase II for solid tumors), and Tempostatin (phase II for bladder cancer).
27. R. Venkatesh, M.J. Ramaiah, H.K. Gaikwad, S. Janardhan, R. Bantu, L. Nagarapu, G.N. Sastry, A.R. Ganesh, and M. Bhadra, *Eur. J. Med. Chem,* **2015**, *94*, 87.
28. R. Venkatesh, K. Suresh, H.K. Gaikwad, S. Janardhan, R. Bantu, L. Nagarapu, G.N. Sastry, and S.K. Banerjee, *Eur. J. Med. Chem*, **2015,** *96*, 22.
29. L. Nagarapu, H.K. Gaikwad, R. Bantu, and S.R. Manikonda, *Eur. J. Med. Chem*, **2011,** *46***,** 2152.
30. L. Nagarapu. H.K. Gaikwad, R. Bantu, K.M. Manda, and S.V. Kalivendi, *Med. Chem. Res*, **2013,** *22*, 165.
31. B. Yadagiri, U.D. Holagunda, R. Bantu. L. Nagarapu, C.G. Kumar, S.U. Pombala, and B. Sridhar, *Eur. J. Med. Chem***, 2014,** *79*, 260.
32. S. Janardhan, P. Srivani, and G.N. Sastry. QSAR and Combinatorial Science, **2006,** ***25***, 860.
33. S. Janardhan, P. Srivani, and G.N. Sastry, *Curr. Med. Chem*, **2006,** *13*, 1169.
34. P. Badrinarayan and G.N. Sastry. Comb. Chem. High Throughput Screen **2011,** *14*, 840.
35. A.S. Reddy, S.P. Pati, P.P. Kumar, H.N. Pradeep, and G.N. Sastry. Curr. Protein Pept. Sci. **2007,** *8*, 329.
36. F. Denizot and R. Lang, *J. Immunol. Meth*. **1986,** *89*, 271.
37. M.C. Alley, D.A. Scudiere, A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, and M.R. Boyd. Cancer Res. **1988,** *48*, 589.
38. A.A. Van de Loosdrecht, R.H.J. Beelen, G.J. Ossenkoppele, M.G. Broekhoven, and M.M.A.C. Langenhuijsen. *J. Immunol. Meth*. **1994,** *174*, 311.

39. S.K. Lee, B. Cui, R.R. Mehta, A.D. Kinghorn, and J.M. Pezzuto, *Chem. Biol. Interact*. **1998,** *115*, 215.

40. C.R. Guimaraes and M. Cardozo, *J. Chem. Inf. Model*, **2008,** *48*, 958.

41. R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, D.E. Shaw, P. Francis and P.S. Shenkin, *J. Med. Chem*, **2004,** *47*, 1739.

Research highlights

- Novel sulphamide tethered quinazolinones hybrids were efficiently synthesized.
- Compounds **5a-j**, **6a-g** screened in viability assays against human cancer cell lines.
- **5c** Showed potent cytotoxicity against four cancers cell lines with GI_{50} 0.09 to 0.21 μM.
- **5i**, **6f** Showed cytotoxic against cancer cell lines with GI_{50} 0.045 to 0.35 μM.
- **5c** Showed Binding mode and identified key active site residues in HDAC8 and EHMT2 proteins.