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Synthesis and antiproliferative assay of triazolyl-2,2-dimethyl-3-phenylpropanoates as potential HDAC inhibitors

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Recently, histone deacetylase (HDAC) inhibition has gained great importance in cancer treatment. We herein, describe the design, synthesis and biological testing of 16 compounds based on the structure modification of methyl 3-(4-(2-chloroacetamido)phenyl)-3-hydroxy-2,2-dimethylpropanoate (**5**) and methyl 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanoate (**14**) as potent HDACIs. Two series were synthesized based on the structure of 3-(4-(2-chloroacetamido)phenyl)-3-hydroxy-2,2-dimethylpropanoate and 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanoate. The compounds were tested *in vitro* for their antiproliferative activity against HeLa cells. The results identified compounds **16b**, **16c**, **18** (IC_{50}; 11.69, 0.69, 3.39 μM respectively) as potential good inhibitors compared to the standard drug doxorubicin (IC_{50}; 2.29 μM). Those compounds also exhibited promising activity against other cancer cell lines namely; HCT-116, MCF-7, PC3, A549 and therefore were selected as hits for further optimization. The docking experiment results performed on the HDAC-2 crystal structure were in close agreement with the biological testing results which suggest that those compounds potentially work through HDAC inhibition.

Introduction

Cancer is one of the major health challenges worldwide. Cancer is an abnormal cell growth that shifts the controlled mechanisms of cell proliferation and differentiation associated with high mortality rate.[1] Chemotherapy is one of the most effective approaches used to treat solid as well as hematological tumors.[2,3] Quinazoline sulfonamide and quinazolindione[4,5] are well established privileged structures in cancer chemotherapy that showed great activity against different types of cancer including breast cancer and pancreatic cancer. Acetylation of histones in a eukaryotic cell is involved in many cellular functions including proliferation, cell-cycle regulation and apoptosis.[6] Histone acetylation is controlled by a balance between histone acetyl transferases (HATs) (associated with gene transcription) and histone deacetylase (HDAC) involved in deacetylation of ε-amino groups of lysine residue on histone tails (associated with gene silencing).[7] Abnormal alterations in the histone acetylation process are associated with cancer development.[8] Therefore, histone deacetylase (HDAC) inhibition is considered one of the promising strategies for the development of novel anticancer agents.[9] HDACIs are classified into different classes depending on their structures namely aliphatic acids, hydroxamic acids, 2-aminoanilides, cyclic peptides, and electrophilic ketones.[10] In general, most of the currently reported HDACIs consist of a zinc-binding group (ZBG) and a five- to six-carbon hydrophobic spacer attached to a hydrophobic group *via* a connection unit (Fig. 1a).[11]

Until now, four HDACIs are approved for cancer treatment including three synthetic hydroxamate HDACIs (SAHA, Belinostat PXD 101, and Panobinostat) and one natural product cyclic depsipeptide (romidepsin).[12] Other compounds in clinical trials include hydroxamic acid derivatives such as panobinostat (LBH589) and pracinostat (SB-939) with an *N*-hydroxyacrylamide moiety exhibiting excellent HDAC inhibitory activity[13,14] and benzamides such as entinostat (MS-275) and mocetinostat (MGCD-0103).[15,16] Therefore, developing and identification of potent HDACIs is a promising therapeutic strategy in cancer treatment.

Our proposed HDAC inhibitors were designed to combine the most important structural features reported for HDAC in a simple chemical structure (Fig. 1b). The compounds contain a sterically hindered amide, ester or hydroxamate on one side of the molecule. A triazole moiety was incorporated for steric effect

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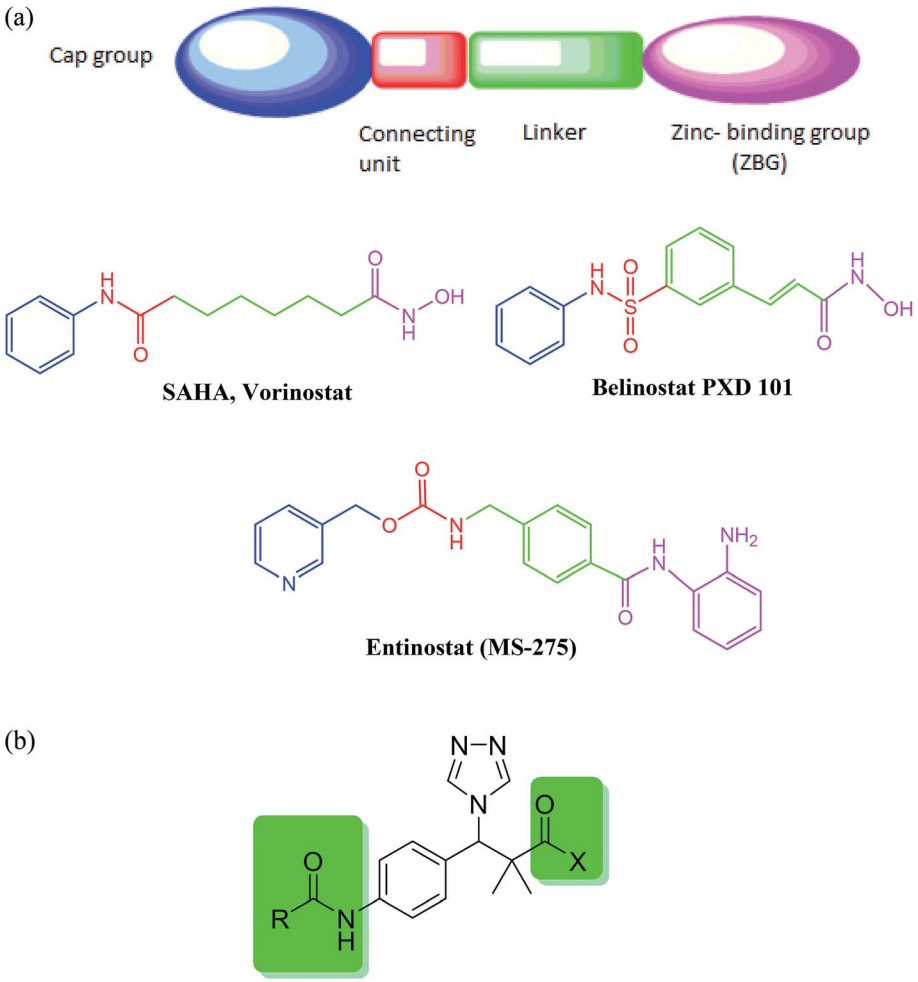


Fig. 1 (a) Classical histone deacetylase (HDAC) inhibitors. (b) Structure design of our proposed HDAC inhibitors.

as well as to optimize the binding interaction and improve the pharmacokinetic properties. A central aromatic ring connects the amide group with another acetamide moiety to mimic the well-known benzamide inhibitors.

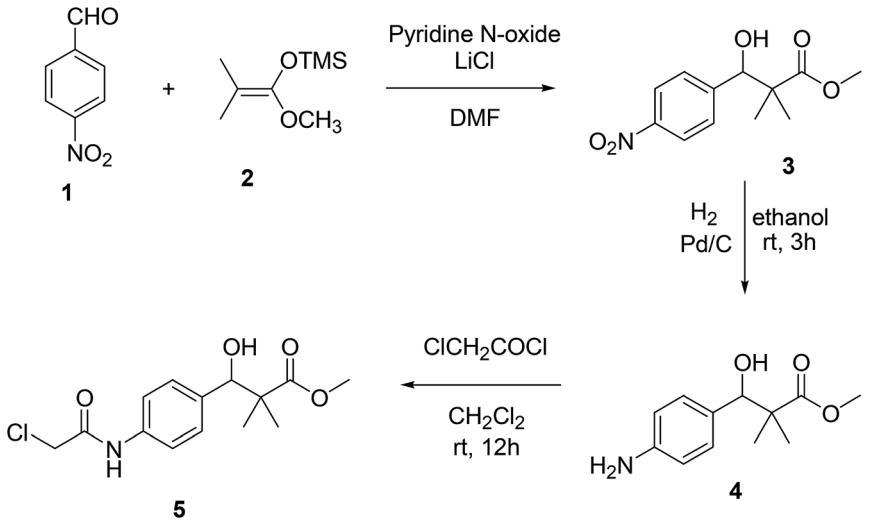
Discussion

We now report the synthesis of the targeted structures; *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**8**), *N*-hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino)acetamido)phenyl)-2,2-dimethyl-3-(4*H*-1,2,4-riazol-4-yl)propanamide (**11**) and their analogues **16a–d**, **18** as HDAC inhibitors.

Methyl 3-hydroxy-2,2-dimethyl-3-(4-nitrophenyl)propanoate (**3**) was prepared by the reaction of 4-nitrobenzaldehyde **1** with trimethylsilyl ketene acetal **2** in the presence of pyridine-*N*-oxide and LiCl in DMF at room temperature under nitrogen atmosphere to afford the ester **3** in 69% yield.[17,18] Reduction of **3** by using hydrogen atmosphere in the presence of Pd/C catalyst in ethanol at room temperature for 3 h afforded the desired ester **4** in 87% yield. The acylation reaction of the ester **4** with chloroacetyl chloride in the presence of triethyl amine and catalytic amount of DMAP in dichloromethane for 12 h. Afforded methyl 3-(4-(2-chloroacetamido)phenyl)-3-hydroxy-2,2-dimethylpropanoate **5** in 70% yield, Scheme 1.

Structure modification of chloroacetamido derivative **5** could simply be achieved by the reaction with nucleophiles and the attachment of triazole moiety to produce both targeted structures *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**8**) and *N*-hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino)acetamido)phenyl)-2,2-dimethyl-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**11**). Thus, the reaction of chloroacetamide derivative **5** with aniline in pyridine under reflux condition for 10 h. Afforded methyl 3-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl) propanoate (**6**) in 84% yield. Successful attachment of triazole residue was achieved by the reaction of **6** with triazole in the presence of CDI in acetonitrile under reflux condition for 48 h. To afford the triazole derivative **7** in 67% yield. The first targeted compound *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**8**) was finally produced in 58% yield by the reaction of **7** with hydroxyl amine hydrochloride in the presence of KOH in ethanol under reflux condition, Scheme 2.

Similarly, the second targeted molecule *N*-hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino)acetamido)phenyl)-2,2-



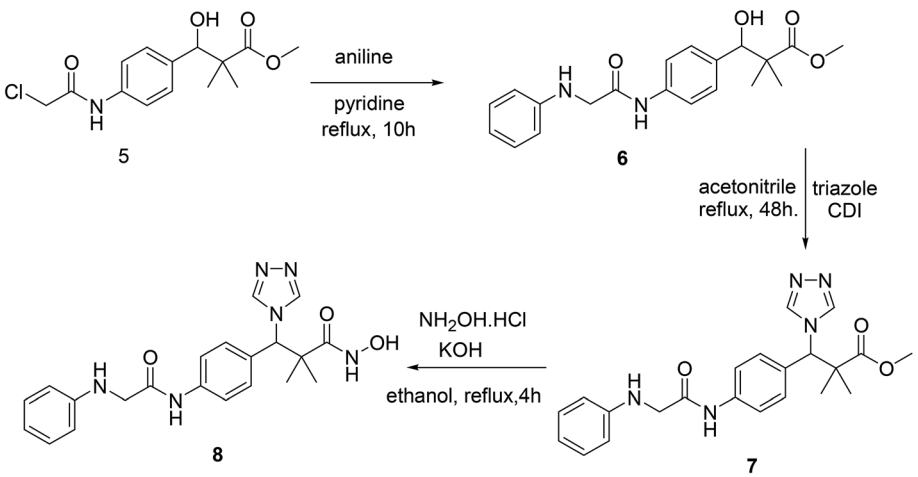
Scheme 1 Sequential synthesis of methyl 3-(4-(2-chloroacetamido)phenyl)-3-hydroxy-2,2-dimethylpropanoate **5**.

dimethyl-3-(4*H*-1,2,4-triazol-4-yl)propan-amide (**11**) is prepared from chloroacetamido derivative **5** *via* three step sequential reactions. Thus, **5** reacted with glycine methyl ester hydrochloride in pyridine under reflux condition for 10 h. Afforded methyl 3-hydroxy-3-(4-(2-((2-methoxy-2-oxoethyl)amino)acetamido) phenyl)-2,2-dimethylpropanoate (**9**) in 71% yield. Next, the reaction of **9** with triazole in the presence of CDI in acetonitrile under reflux condition for 48 h. Afforded **10** in 71% yield and finally **11** was produced in 74% yield by the reaction of **10** with hydroxyl amine hydrochloride in the presence of KOH in ethanol under reflux condition, Scheme 3.

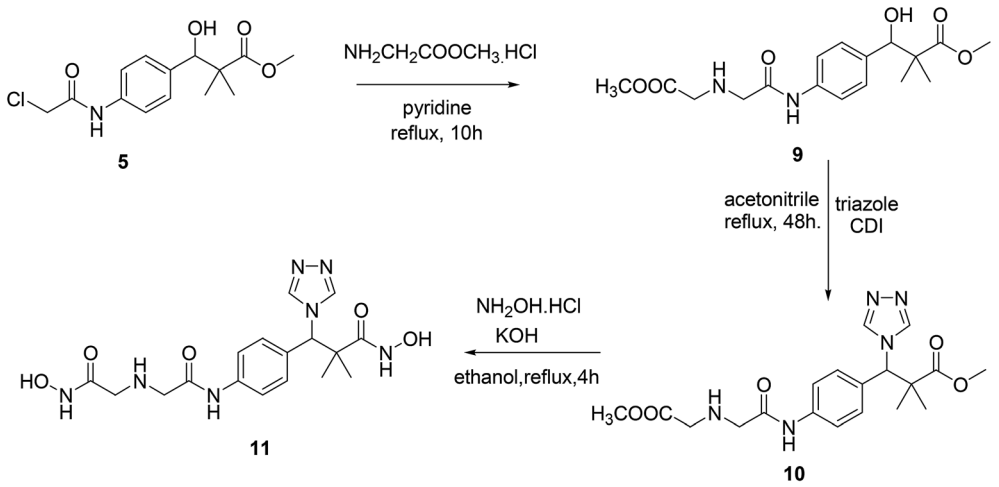
The structure assignment of both targeted structures *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**8**) and *N*-hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino)acetamido)phenyl)-2,2-dimethyl-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**11**) is based on ^{1}H and ^{13}C NMR spectroscopy and physicochemical analysis. Thus, the ^{1}H NMR spectrum of **11** exhibit signals at δ 6.10 and 9.00 ppm are typically associated with two NH groups. The ^{1}H NMR spectrum also presented an important signal at 10.15 ppm that is typically associated with 2 OH oximes. The ^{13}C NMR spectrum displayed signals at $\delta = 49.4$, 52.0, 173.8 and 174.9 ppm, associated with two CH_2 and 2 CO groups, respectively (Fig. 2).

Our next target was the preparation of a number of 3-(4-chlorophenyl)-*N*-arylidene-2,2-dimethylpropanehydrazide **16a–d** and *N*-benzyl-3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanamide (**18**) as structure analogues of compounds **8** and **11** for the CYP26 inhibitors evaluation. The reaction of 4-chlorobenzaldehyde (**12**) with methyl 2-bromo-2-methylpropanoate (**13**) in benzene under reflux condition afforded 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanoate (**14**) in 79% yield. Hydrazinolysis of ester **14** afforded the hydrazide **15**. The reaction of hydrazide with aldehyde in ethanol under reflux condition for 6 h. Afforded the hydrazone **16a–d**, Scheme 4.

On the other hand, the hydrazide reacted with benzyl amine under azide coupling condition to afford *N*-benzyl-3-(4-



Scheme 2 Sequential synthesis of the targeted molecule *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**8**).



Scheme 3 Sequential synthesis of the targeted molecule *N*-hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino)acetamido)phenyl)-2,2-dimethyl-3-(4*H*-1,2,4-triazol-4-yl)propanamide (**11**).

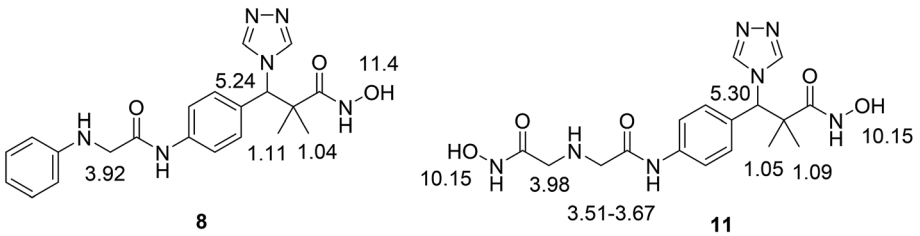


Fig. 2 Selected ^{1}H and ^{13}C NMR spectral data of *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(4*H*-1,2,4-triazol-4-yl) propanamide (**8**) and *N*-hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino)acetamido)phenyl)-2,2-dimethyl-3-(4*H*-1,2,4-triazol-4-yl) propanamide (**11**).

chlorophenyl)-3-hydroxy-2,2-dimethylpropanamide (**18**) in 77% yield, Scheme 5.

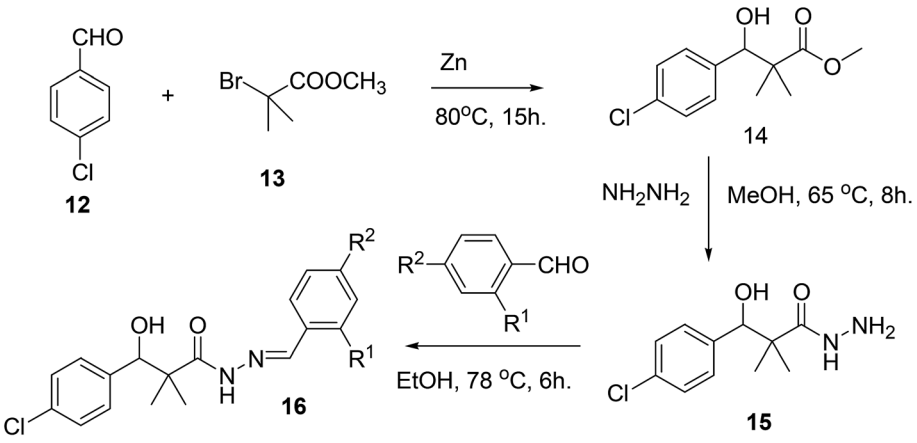
The structure assignment of 3-(4-chlorophenyl)-*N*-arylidene-2,2-dimethylpropanehydrazide **16a–d** and *N*-benzyl-3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanamide (**18**) is based on ^{1}H and ^{13}C NMR spectroscopy and physicochemical analysis. Thus, the ^{1}H NMR spectrum of **16a** showed newly introduced salicylaldehyde signals at 8.59 and 11.40 for CH= and OH groups. *N*-Benzyl-3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanamide (**18**) was identified by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum showed newly introduced benzyl group signals at 4.35 and 6.08 for CH_2 and NH groups. The ^{13}C NMR spectrum displays signals at 46.2 corresponding to CH_2 group.

The cytotoxicity of each compound was investigated against different cancer cell lines namely; HCT-116, MCF-7, PC3, A549 at concentrations (0.1, 1, 10, and 100 μM) using MTT assay colorimetric assay. Data illustrated in (Table 1) shows the percentage of viability of HCT-116 cells after 48 h from treatment with different concentrations of the compounds *versus* control. The results revealed that the compound **16c** had the lowest IC_{50} value (0.69 μM), followed by compounds **16b** and **18** with IC_{50} values equal 11.6 μM, 3.39 μM, respectively compared to the standard drug doxorubicin (IC_{50}; 2.29 μM) (Table 2).

Interestingly, the results of the different concentrations antiproliferative assay conducted on other cancer cell lines were consistent with the HeLa cells results, with compounds **16b**, **16c**, **18** on top of the activity list and also showing close activity to the standard drugs doxorubicin and 5-fluoro uracil (Tables 3–6).

The accurate IC_{50} results on A549 cells again proved compounds **16b** and **16c** as superior antiproliferative agents with IC_{50} equal to 79.4 μM and 85.1 μM respectively compared to an IC_{50} of 72.4 μM for the standard drug 5-fluoro uracil.

The consistent comparable activity of compounds **16b** and **16c** on all tested cell lines suggested that the triazole ring is not of great importance compared to the hydroxyl group. Moreover, shifting the benzyl amide group next to the hydroxy isobutyl group resulted in a highly active compound (compound **18**) possibly due to an increase in the stabilization of the amide bond that is essential for HDAC inhibition. Surprisingly, compound **18** although is the most different in structure among the designed and synthesized compounds as it lacks the triazole, the ester or the hydroxamic acid groups and the benzamide substituents, is one of the most active compounds against almost all tested cancer cell lines. This implies that for our compounds the structurally hindered hydroxyisobutyl acetamide is more important than the benzamide and the hydroxamate moieties. Therefore, compound **18** is considered as



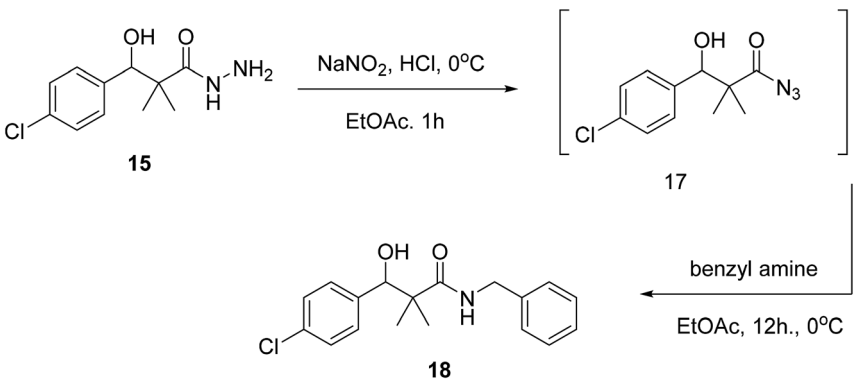
16	R^1	R^2
a	H	F
b	H	$N(CH_3)_2$
c	H	OH
d	OH	OH

Scheme 4 Sequential synthesis of 3-(4-chlorophenyl)-*N*-arylidene-2,2-dimethylpropanehydrazide **16a–d**.

a potential lead for further optimization and development followed by compounds **16b** and **16c** which are basically amino-hydrazone derivatives of compound **18** (Table 7).

The activity of the docked compounds was investigated by comparison of their ligand–receptor interactions with the amino acids of the receptor binding site. As shown in Table 8 and Fig. 3, the original ligand forms three hydrogen bonds with Tyr 308, His145, and Gly154. Moreover, it forms two van der Waals interactions with His 183 and Phe 155. Compound **15** forms six hydrogen bonds with Tyr 308, Asp 269, His 183, His 145 and Asp 181, and it forms one van der Waals interaction with Arg 39. Compound **16a** forms three hydrogen bonds with His 145 and His 183, and it forms two van der Waals interactions with Phe 155 and Arg 39. Compound **16b** forms five hydrogen bonds with Tyr 308, Asp 269, His 146, Asp 181 and His 145, and it forms three van der Waals interactions with Phe 155, His 183 and Arg 39. Compound **16c** forms three hydrogen bonds with Ala 141 and Gly 154, and it forms two van der Waals interactions with Phe 155 and His 183. Compound **16d** forms three hydrogen bonds with Asp 104, Asp 269, and Tyr 308 and it forms two van der Waals interactions with His 145 and Gly 154. Compound **18** forms four hydrogen bonds with Gly 154, Tyr 308, His 146 and His 145, and it forms two van der Waals interactions with Phe 155 and Arg 39.

From the above discussion, compound **18** is the only compound that was able to bind the three key residues that are involved in binding the co-crystallized ligand through hydrogen bonding namely; His 145, Gly 154, and Tyr 308. Compound **16B** formed hydrogen bonding with only two of the key residues namely; His 145, and Tyr 308. This is in close agreement with the antiproliferative assay results that identified compounds **18**, **16B**, **16C** as the most active compounds. Therefore, based on



Scheme 5 Sequential synthesis of *N*-benzyl-3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanamide (**18**).

Table 1 Anticancer activity of tested compounds against HeLa cells

% of cell growth treated with different (conc. μM± SEM) of cpds.				
Cpds.	0.1	1	10	100
3	61.47361	64.73607	64.99267	48.42375
4	57.14809	61.58358	51.6129	44.17155
5	79.98534	90.72581	100.2933	71.66422
6	62.86657	68.58504	75.80645	50.62317
7	94.72141	86.91349	70.96774	49.41349
8	54.80205	54.72874	59.12757	49.81672
9	64.03959	73.05718	62.79326	49.59677
10	94.72141	86.91349	70.96774	49.41349
11	92.33871	93.8783	86.36364	48.86364
14	79.98534	90.72581	100.2933	71.66422
15	62.86657	68.58504	75.80645	50.62317
16a	65.9824	61.10704	58.5044	50.73314
16b	51.46628	53.07918	50.18328	47.83724
16c	52.84311	49.85924	51.64956	53.00587
16d	67.88757	63.57404	71.86435	50.3476
18	53.55572	53.81232	47.54399	86.40029
5-FU	98.05718	77.52933	78.95894	80.42522
Dox	73.24047	70.01466	16.31232	21.04106

Table 2 IC_{50} of the tested compounds against HeLa cells

Compd.	IC_{50} (μM)
3	ND
4	13.2
5	100
6	77.6
7	77.5
8	81.3
9	85.1
10	ND
11	ND
14	ND
15	ND
16a	ND
16b	11.6
16c	0.69
16d	ND
18	3.39
5-FU	ND
Dox	2.29

the comparative docking with the co-crystallized ligand compound **18** is considered a potential hit for HDAC inhibition followed by compound **16B** with rooms for pharmacokinetic and target interaction optimization.

Conclusion

In conclusion, efficient and very simple methods for the synthesis of various HDAC inhibitors were executed. Some of the synthesized compounds exhibited promising anti-proliferative activity against various cancer cell lines namely; HCT-116, MCF-7, PC3, A549. Compounds **16b**, **16c**, **18** were consistently the most active on all tested cells and showed comparable activities to the standard drugs doxorubicin and 5-fluoro uracil. Compound **18** was selected as a potential lead for further optimization and development due to its interesting structural features that differs from other members of the series. The docking experiments performed substantiated the biological testing results and identified compound **18** as a potential HDAC inhibitor.

Table 3 Anticancer activity of tested compounds against HCT-116 cells

% of cell growth treated with different (conc. μM± SEM) of cpds.				
Cpds.	0.1	1	10	100
3	74.25979	70.00955	73.87775	69.91404
4	86.67622	75.45368	80.70678	65.52053
5	80.09531	68.47507	66.93548	54.43548
6	92.33871	93.8783	86.36364	48.86364
7	74.70833	85.58333	88.41667	86.875
8	71.82426	73.20917	69.532	64.51767
9	74.88061	74.11652	78.08023	62.89398
10	75.02388	72.34957	77.31614	57.06781
11	76.69532	71.58548	69.81853	73.68672
14	73.35244	69.67526	67.95606	68.33811
15	72.6361	69.62751	71.48997	70.63037
16a	78.12798	83.52436	81.27985	79.36963
16b	58.11843	61.7001	49.37918	47.94651
16c	64.89971	62.89398	57.02006	55.44413
16d	60.875	64.41667	64.79167	60.08333
18	63.32378	65.85482	68.62464	65.42502
5-FU	73.35244	77.93696	69.43649	62.98949
Dox	82.56925	81.3276	65.234	82.52149

Experimental

General

Solvent were purified and dried by standard procedures. The boiling range of the petroleum ether used was 40–60 °C. Thin

Table 4 Anticancer activity of tested compounds against MCF-7 cells

% of cell growth treated with different (conc. μM± SEM) of cpds.				
Cpds.	0.1	1	10	100
3	89.1342	78.44568	99.3658	97.3465
4	98.1562	94.49858	105.4714	93.839
5	105.6513	109.1291	90.90091	91.14076
6	77.58333	76.16667	72.91667	46.20833
7	88.04348	108.8969	112.4396	90.49919
8	95.57787	93.839	91.3806	78.48898
9	98.39604	98.69585	104.3322	82.44641
10	114.7054	113.806	116.6242	79.50832
11	108.4695	106.8505	102.1736	109.3089
14	113.4463	102.1736	104.0923	99.77515
15	103.9125	104.5121	98.51596	98.33608
16a	111.5875	113.0265	109.5488	93.89897
16b	83.2259	85.56438	77.10988	60.32079
16c	78.66887	80.70754	73.09249	70.63409
16d	60.875	64.41667	64.79167	60.08333
18	102.6533	105.6513	102.4134	106.9105
5-FU	104.632	103.013	92.81967	68.53545
Dox	108.9492	115.1851	84.06536	84.42512

Table 5 Anticancer activity of tested compounds against PC3 cells

% of cell growth treated with different (conc. μM± SEM) of cpds.				
Cpds.	0.1	1	10	100
3	75.70833	70.70833	71.29167	67.91667
4	83.20833	69.66667	69	149.3333
5	78.08333	71.54167	73.58333	123.8333
6	98.39604	98.69585	104.3322	82.44641
7	85.74879	84.46055	87.39936	87.56039
8	66.75	65.66667	67.08333	57.70833
9	77.58333	76.16667	72.91667	46.20833
10	77.33333	80.83333	72.20833	47.25
11	72.41667	77.91667	71.58333	66.58333
14	74.70833	85.58333	88.41667	86.875
15	62.125	66.5	67.375	61.29167
16a	89.1342	78.44568	99.3658	97.3465
16b	66.20833	66.5	53.83333	44.08333
16c	70.5	63.54167	55.08333	46.29167
16d	81.11916	77.89855	75.60386	75.36232
18	59.45833	59.66667	51.20833	45.16667
5-FU	66	62.25	49	50.16667
Dox	63.25	65.16667	49.875	49.04167

Table 6 Anticancer activity of tested compounds against A549 cells

% of cell growth treated with different (conc. μM± SEM) of cpds.				
Cpds.	0.1	1	10	100
3	78.55572	82.36804	70.41789	59.45748
4	80.43478	95.04831	90.05636	72.98712
5	84.29952	83.49436	85.82931	80.47504
6	88.88889	91.38486	78.7037	66.86795
7	60.875	64.41667	64.79167	60.08333
8	88.04348	108.8969	112.4396	90.49919
9	100.7246	94.40419	93.67955	75.60386
10	88.88889	91.38486	78.7037	66.86795
13	93.84058	87.39936	97.70531	65.29791
14	88.28502	87.56039	94.68599	92.3913
15	85.74879	84.46055	87.39936	87.56039
16a	89.1342	78.44568	99.3658	97.3465
16b	98.59098	86.95652	96.53784	46.61836
16c	91.10306	97.26248	110.1449	46.37681
16d	85.74879	84.46055	87.39936	87.56039
18	97.38325	93.27697	94.36393	43.19646
5-FU	79.99195	76.24799	73.59098	66.74718
Dox	79.99195	62.72142	65.25765	73.79227

layer chromatography (TLC): silica gel 60 F254 plastic plates (E. Merck, layer thickness 0.2 mm) detected by UV absorption. Elemental analyses were performed on a Flash EA-1112 instrument at the Microanalytical laboratory, Faculty of Science, Suez Canal University, Ismailia, Egypt. Melting points were determined on a Buchi 510 melting-point apparatus and the values are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively (Bruker AC 400) in $CDCl_3$ and DMSO solution with tetramethylsilane as an internal standard. The NMR analyses were performed at Faculty of Science, Sohag University. The mass spectra were measured with a KRATOS analytical compact; on MALDI-MS the spectrometer was using 2,5-dihydroxy benzoic acid (DHB) as matrix. The starting compound **3** were obtained as described in literature.[17,18]

3-Hydroxy-2,2-dimethyl-3-(4-nitrophenyl)-propionic acid methyl ester (3). To an anhydrous stirred solution of pyridine-*N*-oxide (0.19 g, 0.20 mmol) and LiCl (0.17 g, 0.40 mmol) in DMF (20 ml) were added 4-nitrobenzaldehyde **1** (3.0 g, 20.0 mmol) and trimethylsilyl ketene acetal **2** (5.25 ml, 26.0 mmol) at room temperature under a nitrogen atmosphere. After stirring overnight, the reaction was quenched by the addition of 1 N HCl (5 ml), the product was extracted with ethyl acetate. The organic layer was washed with water, brine and evaporated to dryness, the product was then purified by flash column chromatography (petroleum ether–ethyl acetate) to give 3-hydroxy-2,2-dimethyl-3-(4-nitrophenyl)-propionic acid methyl ester (**3**). 3.47 g, yield 69% white powder. Mp 188–191 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.15 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 3.42 (s, 1H, OH), 3.78 (s, 3H, OCH_3), 5.04 (s, 1H, CH), 7.51 (d, J = 8.4 Hz, 2H, Ar–H), 8.20 (d, J = 8.3 Hz, 2H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 19.23, 22.8 (CH_3), 47.7 (C), 52.4 (OCH_3), 77.8 (CH), 122.9, 128.6, 147.3, 147.5 (C–Ar), 177.7 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 276 $(M + Na)^+$. Found, %: C, 57.12; H, 6.27; N, 5.32. For $C_{12}H_{15}NO_5$ (253.3). Calculated, %: C, 56.91; H, 5.97; N, 5.53.

3-(4-Aminophenyl)-3-hydroxy-2,2-dimethylpropionic acid methyl ester (4). Pd/C catalyst (100 mg) was added to a solution of **3** (1.0 g, 4.0 mmol) dissolved in ethanol (20 ml) and then the reaction was stirred under H_2 atmosphere. After 3 h the hydrogen balloon was removed and the mixture was filtered through Celite, the solvent was then removed under reduce pressure and the oil formed was extracted with methylene chloride (100 ml), washed with water and dried over sodium sulphate, filtered and evaporated in vacuum, the product was then obtained without further purification to give 3-(4-aminophenyl)-3-hydroxy-2,2-dimethylpropionic acid methyl ester (**4**). 0.87 g, yield 87%, yellow powder. Mp 130–132 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 0.90 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 3.58 (s, 3H, OCH_3), 4.65 (s, 1H, OH), 4.93 (s, 2H, NH_2), 5.17 (s, 1H, CH), 6.50 (d, J = 7.9 Hz, 2H, Ar–H), 6.92 (d, J = 7.9 Hz, 2H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 18.5, 19.4 (CH_3), 47.9 (C), 51.3 (OCH_3), 76.8 (CH), 112.8, 128.0, 128.6 (C–Ar), 176.7 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 246 $(M + Na)^+$. Found, %: C, 64.78; H, 7.92; N, 6.09. For $C_{12}H_{17}NO_3$ (223.3). Calculated, %: C, 64.55; H, 7.67; N, 6.27.

Methyl 3-(4-(2-chloroacetamido)phenyl)-3-hydroxy-2,2-dimethylpropanoate (5). To a cold solution (−5 °C), of **4** (2.23 g, 10.0 mmol), triethylamine (1 ml) and DMAP (0.122 g, 1.0 mmol) in methylene chloride (60 ml), was added portion wise under stirring a cold solution (0 °C) of chloroacetyl chloride (1.69 g, 15.0 mmol). After stirring at the same temperature for 30 minutes, the reaction mixture was stirred at room temperature for 12 h The solvent was then removed under reduce pressure, the product was then obtained without further purification to give methyl 3-(4-((2-chloro-2-oxoethyl)amino)phenyl)-3-hydroxy-2,2-dimethylpropanoate (**5**). 2.1 g, yield 70%, white powder. Mp 89–93 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 0.90 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 3.56 (s, 3H, OCH_3), 4.77 (d, J = 3.0 Hz, 2H, CH_2), 5.27 (d, J = 3.0 Hz, 1H, CH)

Table 7 IC_{50} of the tested compounds against A549 cells

Compd.	IC_{50} (μM)
3	ND
4	ND
5	ND
6	ND
7	ND
8	ND
9	ND
10	ND
11	ND
14	ND
15	ND
16A	ND
16b	79.4
16c	85.1
16D	ND
18	89.2
5-FU	72.4
Dox	ND

5.55 (s, 1H, OH), 7.20–7.37 (m, 4H, Ar–H), 8.70 (bs, 1H, NH). ^{13}C-NMR (100.0 MHz, $CDCl_3$), δ, ppm: 20.0, 22.5 (CH_3), 42.4 (C), 46.1 (CH_2), 55.3 (OCH_3), 75.3 (CH), 127.3, 128.5, 130.1, 136.6, 138.9 (C–Ar), 171.0, 173.3 (2 CO). MS (MALDI, positive mode, matrix DHB): m/z = 322.0 $(M + Na)^+$. Found, %: C, 55.79; H, 6.33; N, 4.89. For $C_{14}H_{18}ClNO_4$ (299.8). Calculated, %: C, 56.10; H, 6.05; N, 4.67.

Methyl 3-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino) acetamido)phenyl)propanoate (6). A mixture of methyl 3-(4-((2-chloro-2-oxoethyl)amino)phenyl)-3-hydroxy-2,2-dimethyl propanoate (**5**) (2.99 g, 10.0 mmol) and aniline (1.40 g, 15.0 mmol) was refluxed in pyridine (30 ml) for 10 hours. After cooling to room temperature, the solvent was then removed under reduce pressure and the oil formed was extracted with methylene chloride (100 ml), washed with water and dried over sodium sulphate, filtered and evaporated in vacuum, the product was then obtained without further purification to give methyl 3-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido) phenyl)propanoate (**6**). 3.0 g, yield 84%, white powder. Mp 172–176 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.05 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 3.52 (s, 3H, OCH_3), 4.24 (d, J = 3.0 Hz, 2H, CH_2), 5.14 (s, 1H, CH), 5.69 (bs, 1H, OH), 6.08 (bs, 1H, NH), 7.44–8.08 (m, 10H, NH, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 20.2 (CH_3), 22.4 (CH_3), 47.1 (C), 50.4 (CH_2), 53.4 (OCH_3), 75.5 (CH), 127.1, 128.7, 129.0, 129.4, 130.2, 133.9, 136.6, 139.8, 140.9, 153.3 (C–Ar), 168.6, 173.8 (2 CO). MS (MALDI, positive mode, matrix DHB): m/z = 379.0 $(M + Na)^+$. Found, %: C, 67.36; H, 6.52; N, 7.91. For $C_{20}H_{24}N_2O_4$ (356.4). Calculated, %: C, 67.40; H, 6.79; N, 7.86.

Methyl 2,2-dimethyl-3-(4-(2-(phenylamino)acetamido) phenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanoate (7). To a solution of **6** (0.53 g, 1.5 mmol) in anhydrous acetonitrile (20 ml) was added triazole (6.0 mmol) and CDI (3.0 mmol). The mixture was then heated under reflux for 48 h. The reaction mixture was allowed to cool and then extracted with ethyl acetate. The organic layer was washed by water and dried over sodium sulphate, filtered and evaporated under reduce vacuum. The product was purified by flash column chromatography to give methyl 2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(1*H*-1,2,4-triazol-1-yl) propanoate (**7**). 0.4 g, yield 67%, yellow powder. Mp 183–185 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.01 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 3.29 (s, 3H, OCH_3), 4.01 (d, J = 3.0 Hz, 2H, CH_2), 4.86 (s, 1H, CH), 6.36 (bs, 1H, NH), 7.21–7.38 (m, 9H, Ar–H), 8.45 (s, 1H, Ar–H), 8.57 (s, 1H, Ar–H), 9.86 (bs, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 20.0, 22.3 (CH_3), 45.8 (C), 52.3 (CH_2), 53.4 (OCH_3), 69.7 (CH), 127.0, 127.2, 128.8, 130.6, 132.1, 133.9, 145.1 (C–Ar), 166.5, 170.3 (2 CO). MS (MALDI, positive mode, matrix DHB): m/z = 430.0 $(M + Na)^+$. Found, %: C, 65.05; H, 5.88; N, 17.02. For $C_{22}H_{25}N_5O_3$ (407.5). Calculated, %: C, 64.85; H, 6.18; N, 17.19.

***N*-Hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido) phenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanamide (8).** To a solution of **7** (0.41 g, 1.0 mmol) was added to hydroxyl amine hydrochloride (3.0 mmol) and KOH (6.0 mmol). The reaction mixture was refluxed for 4 h, after cooling to temperature the

Table 8 Summarized ligand–receptor interactions of the docked compounds compared to the original ligand

Molecular target (PDB code)	Original ligand			Docked compounds		
	Co-crystallized ligand	Hydrogen bond no.	van der Waals interaction[a]	Compound	Hydrogen bond no.	van der Waals interaction[a]
3MAX	*N*-(4-Aminobiphenyl-3-yl) benzamide	3 Hydrogen bonds with Tyr 308 His145 Gly154	His 183 and Phe 155	**15**	6 Hydrogen bonds with Tyr 308, Asp 269, His 183, His 145 and Asp 181	Arg 39
				16A	3 Hydrogen bonds with His 145 and His 183	Phe 155 and Arg 39
				16B	5 Hydrogen bonds with Tyr 308, Asp 269, His 146, Asp 181 and His 145	Phe 155, His 183 and Arg 39
				16C	3 Hydrogen bonds with Gly 154, and Ala 141	Phe 155 and His 183
				16D	3 Hydrogen bonds with Tyr 308, Asp 269 and Asp 104	His 145 and Gly 154
				18	4 Hydrogen bonds with Gly 154, Tyr 308, His 146 and His 145	Phe 155 and Arg 39

[a] van der Waals interactions are arene–arene interactions or arene–cation interaction.

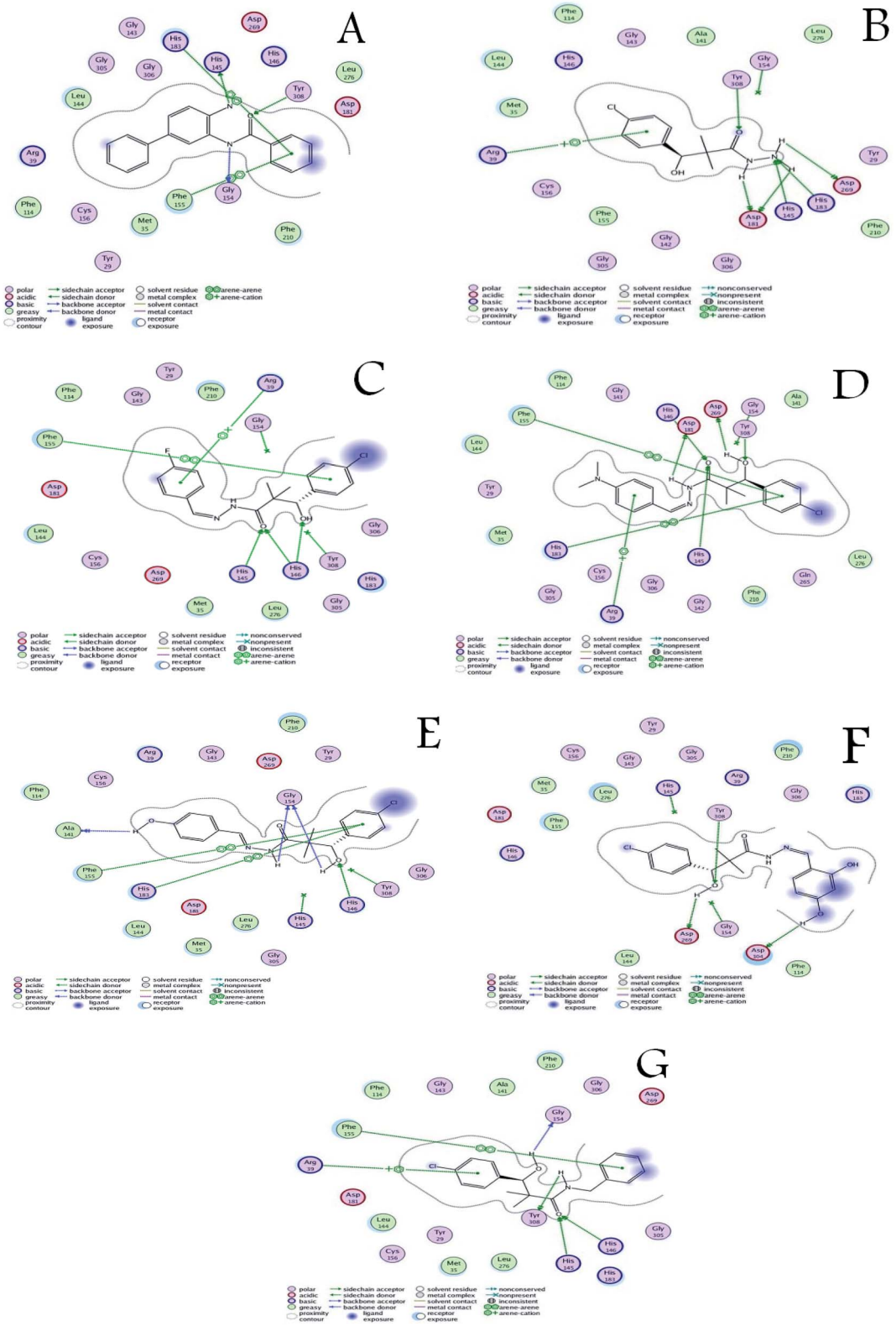


Fig. 3 (A) Ligand–receptor interactions of the original ligand, and (B–G) ligand–receptor interactions of the docked compounds **15**, **16a–d** and **18** respectively.

precipitated was filtered off, washed with ethanol followed by recrystallization from ethanol to give *N*-hydroxy-2,2-dimethyl-3-(4-(2-(phenylamino)acetamido)phenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanamide (**8**). 0.23 g, yield 58%, white crystals. Mp 231–236 °C. ^{1}H NMR spectrum, (400 MHz, DMSO-d$_6$), δ, ppm (J, Hz): 1.04 (s, 3H, CH$_3$), 1.11 (s, 3H, CH$_3$), 3.92 (s, 2H, CH$_2$), 5.24

(s, 1H, CH), 6.74 (bs, 1H, NH), 6.94–7.49 (m, 10H, NH, Ar–H), 8.33 (s, 1H, Ar–H), 8.85 (s, 1H, Ar–H), 11.0 (bs, 1H, NH), 11.4 (bs, 1H, OH). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 21.2, 22.5 (CH_3), 47.1 (C), 52.1 (CH_2), 68.9 (CH), 127.3, 127.7, 129.0, 130.2, 131.6, 132.1, 133.9, 136.2, 145.1 (C–Ar), 167.5, 172.3 (2 CO). MS (MALDI, positive mode, matrix DHB): $m/z = 431.0$ $(M + Na)^+$. Found, %: C, 61.92; H, 5.64; N, 20.09. For $C_{21}H_{24}N_6O_3$ (408.5). Calculated, %: C, 61.75; H, 5.92; N, 20.58.

Methyl 3-hydroxy-3-(4-(2-((2-methoxy-2-oxoethyl)amino) acetamido)phenyl)-2,2-dimethyl propanoate (9). A mixture of 3-[4-(2-chloro-acetylamino)-phenyl]-3-hydroxy-2,2-dimethyl-propionic acid methyl ester (**5**) (2.99 g, 10.0 mmol) and glycine methyl ester hydrochloride (2.50 g, 20.0 mmol) was refluxed in pyridine (30 ml) for 10 hours. After cooling to room temperature, the solvent was then removed under reduce pressure and the oil formed was extracted with methylene chloride (100 ml), washed with water and dried over sodium sulphate, filtered and evaporated in vacuum, the product was crystallized from ethyl acetate–petroleum ether to give methyl 3-hydroxy-3-(4-(2-((2-methoxy-2-oxoethyl)amino)acetamido)phenyl)-2,2-dimethylpropanoate (**9**). 2.5 g, yield 71%, white powder. Mp 79–81 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.07 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 3.57–3.79 (m, 2H, CH_2), 3.83, 3.86 (2 s, 6H, OCH_3), 4.21 (d, $J = 6.0$ Hz, 2H, CH_2), 5.01 (d, $J = 3.0$ Hz, 1H, CH), 5.64 (bs, 1H, OH), 6.38 (bs, 1H, NH), 7.44–7.69 (m, 5H, NH, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 20.5, 22.06 (CH_3), 44.4 (C), 47.0 (CH_2), 49.0 (CH_2), 58.6, 59.0 (2 OCH_3), 75.8 (CH), 127.1, 128.7, 129.0, 129.4, 130.4, 133.9, 136.6 (C–Ar), 160.6, 173.3 (2 CO). MS (MALDI, positive mode, matrix DHB): $m/z = 375.0$ $(M + Na)^+$. Found, %: C, 58.11; H, 7.21; N, 8.03. For $C_{17}H_{24}N_2O_6$ (352.4). Calculated, %: C, 57.94; H, 6.87; N, 7.95.

Methyl 3-(4-(2-((2-methoxy-2-oxoethyl)amino)acetamido) phenyl)-2,2-dimethyl-3-(1*H*-1,2,4-triazol-1-yl)propanoate (10). Prepared *via* reaction of **9** with CDI and triazole, after 48 h reflux, column chromatography (ethyl acetate–petroleum ether) gave this product **10**, yield 71%, yellowish oil. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.04 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 3.53–3.80 (m, 2H, CH_2), 3.83, 3.86 (2 s, 6H, OCH_3), 4.25 (d, $J = 6.0$ Hz, 2H, CH_2), 5.37 (s, 1H, CH), 7.19 (bs, 1H, NH), 7.47–8.45 (m, 7H, NH, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 20.1, 22.3 (CH_3), 45.2 (C), 45.7 (CH_2), 50.0 (CH_2), 54.8, 56.9 (2 OCH_3), 73.3 (CH), 128.5, 129.0, 129.3, 137.2, 139.5, 141.4, 153.5, 154.9 (C–Ar), 170.5, 172.8 (2 CO). MS (MALDI, positive mode, matrix DHB): $m/z = 426$ $(M + Na)^+$. Found, %: C, 56.34; H, 6.67; N, 17.71. For $C_{19}H_{25}N_5O_5$ (403.4). Calculated, %: C, 56.57; H, 6.25; N, 17.36.

***N*-Hydroxy-3-(4-(2-((2-(hydroxyamino)-2-oxoethyl)amino) acetamido)phenyl)-2,2-dimethyl-3-(1*H*-1,2,4-triazol-1-yl) propanamide (11).** Prepared *via* reaction of **10** with hydroxyl amine hydrochloride in alcoholic KOH, after 4 h, stirred, filtration and washed by ethanol gave this product **11**. Yield 74%, white crystals. Mp 154–157 °C. ^{1}H NMR spectrum, (400 MHz, DMSO-d_6), δ, ppm (J, Hz): 1.05 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 3.51–3.67 (m, 2H, CH_2), 3.98 (d, $J = 3.0$ Hz, 2H, CH_2), 5.30 (s, 1H, CH), 6.10 (bs, 1H, NH), 7.19–8.05 (m, 8H, 2 NH, Ar–H), 9.00 (bs, 1H, NH), 10.15 (bs, 2H, 2 OH). ^{13}C-NMR (100 MHz, DMSO-d_6), δ, ppm: 20.0, 21.1 (CH_3), 42.4 (C), 49.4 (CH_2), 52.0 (CH_2), 74.7 (CH), 128.5, 129.0, 129.3, 129.9, 137.2, 139.5, 141.4, 153.6 (C–Ar), 173.8, 174.9 (2 CO). MS (MALDI, positive mode, matrix DHB): $m/z = 428.0$ $(M + Na)^+$. Found, %: C, 50.21; H, 5.52; N, 24.07. For $C_{17}H_{23}N_7O_5$ (405.4). Calculated, %: C, 50.36; H, 5.72; N, 24.18.

Preparation of methyl 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanoate (14). To a solution of 4-chlorobenzaldehyde (**12**) (0.7 g, 5.0 mmol) and Zn (6.0 mmol), in benzene was added methyl 2-bromo-2-methylpropanoate (**13**) (1.0 ml, 5.0 mmol). The reaction mixture was refluxed for 15 h. The reaction mixture was cooled, evaporated under reduced pressure and was then purified by flash column chromatography (petroleum ether–ethyl acetate). 0.96 g, yield 79%, white crystals. Mp 62–63 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.11 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 3.20 (bs, 1H, OH), 3.73 (s, 3H, OCH_3), 4.88 (s, 1H, CH), 7.24 (d, $J = 8.4$, 2H, Ar–H), 7.29 (d, $J = 8.4$, 2H, Ar–H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 18.9, 22.7 (CH_3), 47.5 (C), 52.1 (OCH_3), 77.8 (CH), 127.8, 128.9, 133.4, 138.3 (C–Ar), 177.9 (CO). MS (MALDI, positive mode, matrix DHB): $m/z = 265.0$ $(M + Na)^+$. Found, %: C, 58.92; H, 6.07. For $C_{12}H_{15}ClO_3$ (242.7). Calculated, %: C, 59.39; H, 6.23.

3-(4-Chlorophenyl)-3-hydroxy-2,2-dimethylpropanehydrazide (15). To a solution of methyl 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanoate (**14**) (1.93 g, 8.0 mmol) in methanol (50 ml), hydrazine hydrate (3 ml, 48.0 mmol) was added. The reaction mixture was refluxed for 8 h, after cooling to temperature the precipitated hydrazide was filtered off, washed with water and ethanol followed by recrystallization from aqueous ethanol to give 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanehydrazide (**15**). 1.7 g, yield 88%, white crystals. Mp 96–98 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 0.90 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 4.17 (bs, 2H, NH_2), 4.77 (s, 1H, CH), 5.55 (d, $J = 3.0$ Hz, 1H, OH), 7.25–7.34 (m, 5H, Ar–H + NH). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 19.2, 23.0 (CH_3), 49.1 (C), 78.2 (CH), 127.7, 129.1, 132.6, 136.5, 139.4 (C–Ar), 179.3 (CO). MS (MALDI, positive mode, matrix DHB): $m/z = 265.0$ $(M + Na)^+$. Found, %: C, 54.23; H, 6.18; N, 11.81. For $C_{11}H_{15}ClN_2O_2$ (242.7). Calculated, %: C, 54.44; H, 6.23; N, 11.54.

General procedure for synthesis of 3-(4-chlorophenyl)-*N*-arylid ene-2,2-dimethylpropane hydrazide 16a–d.

A mixture of 3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropane hydrazide (**15**) (2.1 g, 0.90 mmol) and aldehydes (1.0 mmol) were refluxed in ethanol (30 ml) for 6 hours. After cooling to room temperature, the resulting solid was filtered, washed with ethanol and recrystallized from ethanol.

3-(4-Chlorophenyl)-*N*-(4-fluorobenzylidene)-3-hydroxy-2,2-dimethyl propanehydrazide (16a). 2.2 g, ield 73%, yellow crystals. Mp 159–161 °C. ^{1}H NMR spectrum, (400 MHz, DMSO-d_6), δ, ppm (J, Hz): 1.03 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 4.88 (d, $J = 3.0$ Hz, 1H, CH), 5.75 (d, $J = 3.0$ Hz, 1H, OH), 7.33–7.37 (m, 8H, Ar–H), 8.43 (s, 1H, CH), 10.80 (bs, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6), δ, ppm: 20.7, 22.5 (CH_3), 47.3 (C), 76.5 (CH), 116.4, 116.6, 127.8, 129.5, 129.8, 131.0, 131.1,

141.6, 146.2, 160.7, 163.2, 165.6 (CH, C–Ar), 172.6 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 371.0 $(M + Na)^+$. Found, %: C, 62.07; H, 5.53; N, 8.11. For $C_{18}H_{18}ClFN_2O_2$ (348.8). Calculated, %: C, 61.98; H, 5.20; N, 8.03.

3-(4-Chlorophenyl)-*N*-(4-(dimethylamino)benzylidene)-3-hydroxy-2,2-dimethylpropane hydrazide (16b). 2.5 g, yield 80%, white crystals. Mp 220–222 °C. ^{1}H NMR spectrum, (400 MHz, DMSO-d_6), δ, ppm (J, Hz): 1.02 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 3.28 (s, 6H, 2 CH_3), 4.86 (d, J = 3.0 Hz, 1H, CH), 5.72 (d, J = 3.0 Hz, 1H, OH), 6.75–6.78 (m, 2H, Ar–H), 7.30–7.67 (m, 6H, Ar–H), 8.50 (s, 1H, CH), 10.46 (bs, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6), δ, ppm: 20.8, 22.5 (CH_3), 40.2 (2 CH_3), 47.2 (C), 76.5 (CH), 112.2, 112.3, 122.1, 127.8, 128.7, 129.8, 129.9, 132.0, 141.8, 148.3, 151.9, 152.5, 160.2 (CH, C–Ar), 172.1 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 396.0 $(M + Na)^+$. Found, %: C, 64.43; H, 6.66; N, 11.59. For $C_{20}H_{24}ClN_3O_2$ (373.9). Calculated, %: C, 64.25; H, 6.47; N, 11.24.

3-(4-Chlorophenyl)-3-hydroxy-*N*-(2-hydroxybenzylidene)-2,2-dimethylpropanehydrazide (16c). 2.5 g, yield 83%, white crystals. Mp 165–167 °C. ^{1}H NMR spectrum, (400 MHz, DMSO-d_6), δ, ppm (J, Hz): 1.04 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 4.89 (s, 1H, CH), 5.75 (bs, 1H, OH), 6.92 (d, J = 6.0 Hz, 2H, Ar–H), 7.29–7.47 (m, 6H, Ar–H), 8.59 (s, 1H, CH), 11.08 (bs, 1H, NH), 11.40 (bs, 1H, OH). ^{13}C-NMR (100 MHz, DMSO-d_6), δ, ppm: 19.9, 20.6 (CH_3), 40.1 (C), 81.7 (CH), 107.8, 111.1, 111.6, 114.8, 115.6, 121.1, 122.3, 125.3, 128.0, 132.0, 142.5, 144.1, 154.4 (CH, C–Ar), 190.3 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 369.0 $(M + Na)^+$. Found, %: C, 62.63; H, 5.42; N, 8.31. For $C_{18}H_{19}ClN_2O_3$ (346.8). Calculated, %: C, 62.34; H, 5.52; N, 8.08.

3-(4-Chlorophenyl)-*N*-(2,4-dihydroxybenzylidene)-3-hydroxy-2,2-dimethylpropanehydrazide (16d). 2.3 g, yield 70%, white crystals. Mp 241–243 °C. ^{1}H NMR spectrum, (400 MHz, DMSO-d_6), δ, ppm (J, Hz): 1.01 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 4.86 (d, J = 3.0 Hz, 1H, CH), 5.75 (d, J = 3.0 Hz, 1H, OH), 6.30–6.37 (m, 2H, Ar–H), 7.21–7.38 (m, 5H, Ar–H), 8.45 (s, 1H, CH), 9.86 (bs, 1H, NH), 10.86 (bs, 1H, OH), 11.57 (bs, 1H, OH). ^{13}C-NMR (100 MHz, DMSO-d_6), δ, ppm: 20.49, 22.4 (CH_3), 47.2 (C), 76.5 (CH), 103.2, 108.0, 111.0, 127.8, 129.8, 131.9, 132.0, 133.4, 141.6, 149.3, 160.0, 161.0 (CH, C–Ar), 172.1 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 385.0 $(M + Na)^+$. Found, %: C, 59.73; H, 5.43; N, 7.48. For $C_{18}H_{19}ClN_2O_4$ (362.8). Calculated, %: C, 59.59; H, 5.28; N, 7.72.

***N*-Benzyl-3-(4-chlorophenyl)-3-hydroxy-2,2-dimethylpropanamide (18).** To a cold solution (−5 °C), of hydrazide **15** (2.0 g, 8.0 mmol) in acetic acid (60 ml), hydrochloric acid (5 N, 30 ml), was added portion wise under stirring a cold solution (0 °C) of sodium nitrite (0.7 g, 10.0 mmol) in water (30 ml). After stirring at the same temperature for 30 minutes, the *in situ* generated azide **17** was extracted with cold ethyl acetate and washed successively with cold water, 5% $NaHCO_3$ and water. After drying over anhydrous sodium sulphate, the azide **17** was used without further purification in the next step. Benzyl amine (0.90 g, 9.0 mmol) was added to the previously prepared cold dried solution of the azide **17**. Afterwards the mixture was kept 12 h in the refrigerator and then at room temperature for another 12 h. The reaction mixture was washed with 0.1 N HCl, water, 5% $NaHCO_3$ and water then dried over anhydrous sodium sulphate, the solvent was evaporated in vacuum and the residue was crystallized from ethyl acetate–petroleum ether to give **18**. 2.0 g, yield 77%, white crystals. Mp 125–127 °C. ^{1}H NMR spectrum, (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.02 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 3.4 (bs, 1H, OH), 4.35 (d, J = 3.0 Hz, 2H, CH_2), 4.60 (s, 1H, CH), 6.08 (bs, 1H, NH), 7.10–7.28 (m, 9H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ, ppm: 20.7, 22.6 (CH_3), 43.6 (C), 46.2 (CH_2), 60.3 (CH), 127.7, 128.0, 128.3, 128.8, 129.0, 133.5, 137.8, 139.3 (C–Ar), 177.3 (CO). MS (MALDI, positive mode, matrix DHB): m/z = 340.0 $(M + Na)^+$. Found, %: C, 67.96; H, 6.12; N, 4.63. For $C_{18}H_{20}ClNO_2$ (317.8). Calculated, %: C, 68.03; H, 6.34; N, 4.41.

Antiproliferative assay

Materials and methods

Cell cultures. A human lung cancer cell line (A549) was propagated in DMEM medium High Glucose (DMEM High Glucose w/stable Glutamine w/Sodium Pyruvate, Biowest), human breast adenocarcinoma (MCF-7), human prostate cancer (PC3), colon cancer (HCT-116), and cervical cancer (HeLa) were propagated in RPMI-1640 medium L-glutamine (Lonza Verviers SPRL, Belgium, cat#12-604F), both medium were supplemented with 10% fetal bovine serum (FBS) (Seralab, UK, cat# EU-000-H), and 1% antibiotic (Antibiotic antimycotic, Biowest, cat#). All cell lines were purchased from the American Type Culture Collection (ATCC, USA) through VACERA Co.[19] The cells were incubated in 5% CO_2 humidified at 37 °C for growth.

Evaluation of cell proliferation by MTT assay. The cytotoxic effect of the tested compounds on five cancer cell lines was evaluated by the MTT (3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay as reported previously with slight modification.[20,21] In brief, after evaluation of cell count and viability by trypan blue dye, cancer cells (1×10^4 cells per well) were seeded in a 96-well plate in triplicate and were allowed to adhere for 24 h. The tested compounds were dissolved in 500 μl dimethyl sulfoxide (DMSO) to have stock solution of 100 mM, as the final concentration of DMSO in the culture medium never exceeded 0.2% (v/v)[22] and then various concentrations of tested compounds were prepared by further diluting in complete medium to have final concentration of 0.1, 1, 10, and 100 μM. In the next day the medium was replaced with fresh medium with the indicated concentrations of tested compounds and cells were allowed to grow for 48 h. Four hours before completion of incubation, 10 μl of MTT (5 mg mL^{-1} in PBS w/o Ca, Mg, Lonza Verviers SPRL Belgium, cat#17-516F) was added in each well. After completing the incubation, 100 μl of dimethyl sulfoxide (DMSO) was added to each well, then the 96 well plates were centrifuged for 5 minutes at 4000 rpm to precipitate the formazan crystals. Color developed after the reaction was measured at 490 nm using Bio-Tek microplate reader. The experiment was conducted in triplicate.

Data were calculated as percent of cell viability by the following formula: % cell viability = (mean absorbance in test wells/mean absorbance in control wells) × 100.[23]

Molecular modeling studies. All the molecular modeling studies were carried out on Intel® Core™ i3 CPU, 2.40 GHZ processor, and 3 GB memory with Windows 7 operating system

using Molecular Operating Environment (MOE 2008-10 Chemical Computing Group, Canada) as the computational software.

For the docking studies, crystal structure of human HDAC2 complexed with an *N*-(2-aminophenyl)benzamide was obtained from the freely accessible protein data bank (PDB code: 3max), verification process was performed by redocking of the co-crystallized ligand into the active site using the default settings. The compounds under study were constructed 2D using ChemBio-office 2015, converted to 3D by builder interface of MOE program, and then were subjected to energy minimization with MMFF94X force and the partial charges were automatically calculated. Different conformers for each compound are imported by systematic conformational of the MOE and saved in an mdb database file to be docked into the active site of the receptor. Each complex was analyzed for interaction, 2D images were taken by using MOE visualizing tool.

Conflicts of interest

There are no conflicts to declare.

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