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Design, synthesis and anticancer evaluation of tetrahydro-β-carboline-hydantoin hybrids

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ABSTRACT

A series of new tetrahydro-β-carboline-hydantoin hybrids have been designed and synthesized based on the structure of the known Eg5 inhibitor HR22C16. These compounds have been evaluated for their anticancer activity against lung (A549), cervical (ME180, HeLa), prostate (PC-3) and breast (MCF-7) cancer cell lines by MTT assay. These hybrids have displayed significant in vitro cytotoxicity in comparison to etoposide against PC-3, A549, and MCF-7 cell lines. The hybrids **3a**, **3b**, **3c**, **3e**, **3f**, **3g**, **4b**, **4c**, **4e** and **4f** appear to be more effective against the PC-3 cell line, among which compound **4b** displayed the highest cytotoxicity (6.08 ± 0.2, IC_{50} μM). Based on these results, an attempt was made to rationalize their mechanism of action through cell cycle analysis studies. The flow-cytometric analysis of compound **4b** in PC-3 cells indicated a G2/M cell cycle arrest. Molecular docking studies substantiate that these compounds indeed bind to the allosteric site of Eg5 formed from Glu116, Gly117, Glu118, Trp127, Ala133, Ile136, Pro137, Tyr211, Leu214, and Glu215 residues with the most potent compound **4b** showing the most favorable interaction.

Kinesins are ATPases, which hydrolyze ATP to generate energy required for the functioning of microtubules (MTs).[1] The mitotic kinesins are a sub-group of kinesins that act only during the mitotic phase of cell division. Kinesin spindle enzyme (KSP, KIf11, HKSP, KNSL1, TRIP5) or Homo sapiens Eg5 (HsEg5) is a crucial player in the development and function of the mitotic spindle and controls mitosis through bipolar spindle formation and chromosome separation.[2] It belongs to kinesin-5 family or bimC (blocked in mitosis) subfamily of kinesins. Inhibition of this protein results in mono astral spindles which leads to cell cycle arrest and apoptosis without interfering with other microtubule-dependent processes, thus it is a potential target in cancer therapy.[3] KSP came into the limelight as a cancer target in 1999 when the first KSP inhibitor, monastrol, was discovered. Many pharmaceutical companies now have a number of KSP inhibitor drugs in their drug development pipeline.[4]

Disruption of microtubule formation leads to hair-loss, neurotoxicity, weight-loss and other side effects as microtubules are involved in the proper functioning and maintenance of cells. However, Eg5 is significantly over expressed in proliferative tissues (bone marrow, thymus, testis, tonsils) and tumors but not in non-proliferative ones (central nervous system), hence there is less scope for side effects than traditional anti-mitotic drugs which affects normal cells along with cancer cells by disrupting microtubule formation.[5,6]

The β-carboline alkaloids are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido[3,4-*b*]indole ring system.[7] The tetrahydro-β-carboline (THβC) ring is the major skeleton that occurs in nature among many indole alkaloids and marine organisms (Fig. 1a).[8] This ring system has been shown to be effective as anti-cancer,[9] anti-inflammatory,[10] anti-HIV,[11] anti-depressant,[12] and for erectile dysfunction,[13a,b] and possess a range of other biological activities.[14] They have been characterized as a class of potential antitumor agents, which mainly exhibit their antitumor activity through multiple mechanisms, such as intercalating into DNA,[15] inhibiting topoisomerase I and II (Top I and II),[16] Cyclin-Dependant Kinase (CDK),[17] mitogen activated protein kinase-activated protein kinase 2 (MK-2),[18] polo-like kinase-1 (PLK1),[19] I-Kappa-B kinase (IKK)[12] and kinesin Eg5.[20]

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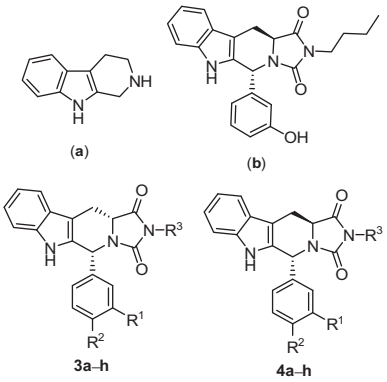


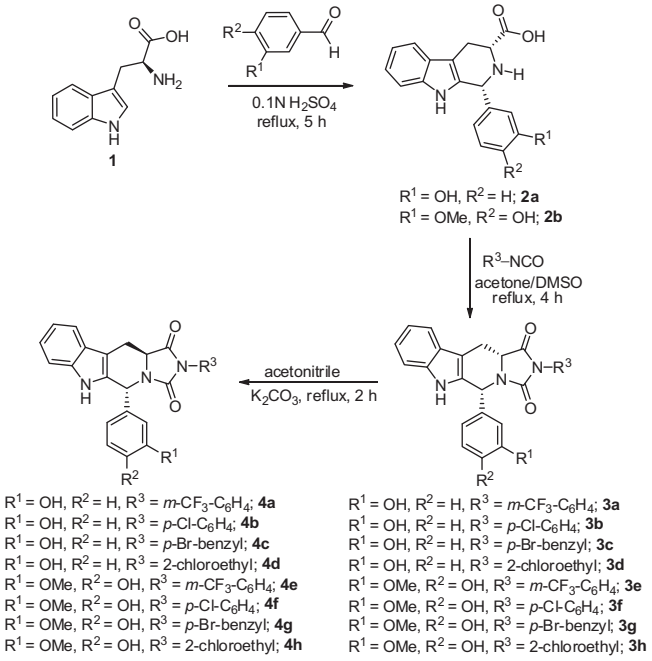
Figure 1. Representative chemical structures of 2,3,4,9-tetrahydro-1*H*-pyrido [3,4-*b*] indole (**a**), HR22C16 (**b**), and newly synthesized tetrahydro-β-carboline-hydantoin hybrids **3a**–**h** and **4a**–**h**.

The hexahydro-imidazo[1,5-*b*]-β-carboline-1,3-dione (HR22C16) derivative (Fig. 1b) was identified as a potent selective inhibitor of Eg5 (IC_{50} = 800 nM)[21] via HTP screening of 16,000 small molecules. HR22C16 was reported as an antimitotic agent that induced cell death in both taxol-sensitive and resistant cancer cells.[22] SAR study of HR22C16 analogs indicated that certain structural features were needed for potent Eg5 inhibition such as two chiral centers with *trans* configuration, carbon 11a (C11a) with *S*-configuration, the 3-hydroxylphenyl group at C5. In continuation of our efforts in the search for novel anticancer compounds, we have designed and synthesized a series of new hydantoin-fused β-carboline hybrids **3a**–**h** and **4a**–**h** as shown in Figure 1.

In the drug discovery arena, introduction of bioactive components into a biologically active natural scaffold provides the means for accessing wider range of biological activity profiles. A simple and complex structural alteration of these active scaffolds aids the development of new drugs with improved therapeutic properties. We have introduced a hydantoin ring on the tetrahydro-β-carboline scaffold, with an aim to develop new potent Eg5 inhibitors. The hydantoin is a useful biological motif and easy to introduce into a scaffold. It is also an important synthetic intermediate, owing to its utility in the preparation of diverse pharmacologically active compounds.

Our synthetic strategy began with the Pictet-Spengler condensation of L-tryptophan (**1**) with aromatic aldehydes under acidic condition which affords tetrahydro-β-carbolines **2a**,**b**[13b] as shown in Scheme 1. The compounds **2a**,**b** were converted into hydantoin-fused tetrahydro-β-carbolines **3a–h** (yields 50–80%) by the reaction with different isocyanates. During this reaction, preferentially *cis* hydantoin-fused tetrahydro-β-carbolines were obtained (reaction time <4 h). Next, these *cis* hydantoin-fused tetrahydro-β-carbolines (**3a–h**) were simply converted into *trans* hydantoin-fused tetrahydro-β-carbolines (**4a–h**) via base-catalyzed epimerization at C11a position, by refluxing in acetonitrile and K_2CO_3. The *cis* and *trans* compounds were characterized through ^{13}C NMR, where the *trans* compounds exhibited comparatively up-field chemical shifts.[23] Stereochemistry was assigned for the representative compounds such as *cis*-**3d** and *trans*-**4d** by detailed NOE studies. All proton assignments were carried out by g-DQFCOSY and NOESY 2D experiments as depicted in Figure 2. In compound **3d**, the '*R*' configuration at position C11a (H1–C) was confirmed by cross peaks between H1–H8, H2–H13 and H2–H9 protons together with the coupling constants J_{H2-H1} = 11.3 Hz and J_{H2a-H1} = 4.3Hz. Similarly, for compound **4d**, the '*S*' configuration at position C11a (H1–C) was assigned by the cross peaks between H1–H9 and H1–H13 protons along with coupling constants J_{H1-H2} = 5.6 Hz and J_{H2a-H1} = 11.0 Hz.

The newly synthesized tetrahydro-β-carboline-hydantoin hybrids were evaluated for their in vitro cytotoxicity against A549



Scheme 1. Synthesis of tetrahydro-β-carboline-hydantoin hybrids **3a**–**h** and **4a**–**h**.

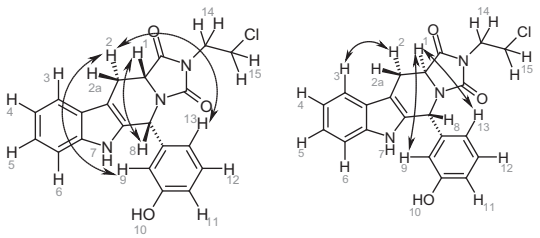


Figure 2. Characteristic NOE diagram for compound **3d** (*cis*) and **4d** (*trans*) (NOEs shown in dotted lines).

(lung adenocarcinoma), ME-180 (cervical carcinoma), HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma), and PC-3 (prostate cancer) cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The screening results for all the compounds were summarized in Table 1. The compounds **3a**, **3b**, **3c**, **3e**, **3f**, **3g**, **4b**, **4c**, **4e** and **4f** are equally active (IC_{50} <20 μM) compared to etoposide against PC-3, A549, and MCF-7 cell lines. Specifically, compounds **3a**, **3b**, **3c**, **3e**, **4b** and **4c** displayed potent cytotoxicity against lung cancer cell lines. Interestingly, the compounds **3a**, **3b**, **3c**, **3e**, **3f**, **3g**, **4b**, **4c**, **4e** and **4f** appear to be more effective against the PC-3 cell lines, among which compound **4b** displays the highest cytotoxicity (6.08 ± 0.2 μM).

On the basis of this cytotoxicity data, the structure-activity relationship (SAR) was analyzed for the synthesized compounds. Compounds **3d**, **3h**, **4d** and **4h** posssessing chloroethyl substituent on the hydantoin ring exhibited marginal or no activity against the different human tumor cell lines. Only compounds **3f** and **3g** with 4-chorophenyl and 4-bromobenzyl groups on hydantoin ring respectively, displayed cytotoxicity against breast cancer cell lines. Compounds **3e–h** and **4e–h** with a 4-hydroxy-3-methoxyphenyl instead of a 3-hydroxylphenyl group at C5-position exhibited a marked decrease or no activity against these cell lines except in the PC-3 cell line. The results convey that the structural features such as two chiral centers with *trans* configuration, the 3-hydroxylphenyl group at C5-position, and the 4-chlorophenyl substituent on the hydantoin ring significantly enhanced the cytotoxicity by

Table 1
In vitro cytotoxicity (IC_{50} μM) against human cancer cell lines

Compound	A549	ME180	HELA	PC-3	MCF-7
3a	40.0 ± 0.91	22.6 ± 3.8	13.6 ± 0.2	11.2 ± 0.7	42.6 ± 5.1
3b	34.8 ± 0.5	17.0 ± 0.4	12.1 ± 0.9	10.3 ± 0.2	40.5 ± 0.7
3c	35.4 ± 0.5	17.5 ± 0.1	11.2 ± 0.7	9.13 ± 1.2	27.9 ± 2.7
3d	>100	48.0 ± 4.6	30.3 ± 0.2	22.3 ± 0.3	>100
3e	43.9 ± 2.8	30.7 ± 1.2	15.6 ± 0.2	11.7 ± 0.6	35.9 ± 0.7
3f	>100	>100	30.6 ± 0.7	13.4 ± 0.7	19.7 ± 0.2
3g	>100	>100	84.3 ± 7.2	12.0 ± 1.5	19.9 ± 1.1
3h	>100	>100	33.1 ± 0.85	29.1 ± 0.8	>100
4a	>100	>100	31.9 ± 0.3	37.12 ± 38	70.9 ± 3.4
4b	16.0 ± 1	24.6 ± 0.9	6.5 ± 0.6	6.08 ± 0.2	26.0 ± 3.0
4c	34.8 ± 0.4	88.6 ± 1.1	24.3 ± .04	8.9 ± 0.1	24.9 ± 3.3
4d	68.0 ± 1.4	59.5 ± 1.8	60.3 ± 0.8	49 ± 1.5	48.2 ± 3.6
4e	>100	27.4 ± 0.7	11.4 ± 0.5	9.6 ± 0.01	48.0 ± 4.0
4f	>100	>100	30.3 ± 1.6	13.4 ± 0.7	42.2 ± 2.6
4g	77.5 ± 1.7	>100	82.9 ± 1.9	>100	>100
4h	>100	>100	50.0 ± 0.9	35.5 ± 0.04	50.7 ± 4.6
Etoposide	50 ± 2.9	8.9 ± 0.3	4.71 ± 1.4	14.4 ± 3.2	23.9 ± 0.3
Doxorubicin	1.5 ± 0.6	0.39 ± 0.01	0.36 ± 0.02	Not tested	Not tested

All the values are mean ± SD, *n* = triplicate wells with triplicate experiments.

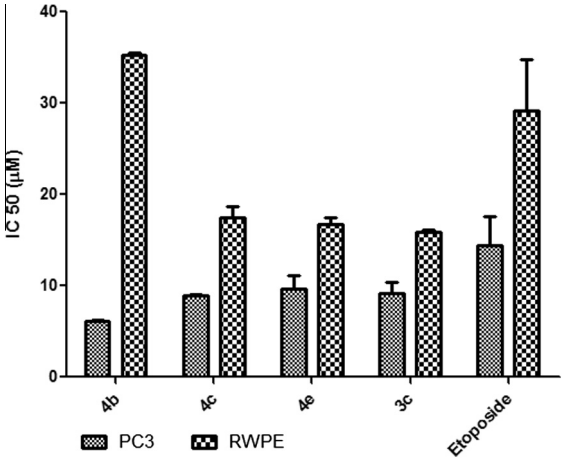


Figure 3. Specificity of compounds **4b**, **4c**, **4e**, **3c** and etoposide toward normal prostate epithelial cells (RWPE) compared to cancer cells.

2.4 and 3.12 fold in PC-3 and A549 cell lines respectively, compared to etoposide.

From the cytotoxicity data, the compounds **4b**, **4c**, **4e** and **3c** were found to be highly potent in PC-3 cell line. Hence, these compounds were further tested for in vitro cytotoxicity on normal prostate epithelial cells (RWPE), to investigate the specificity toward cancer cells. It is interesting to note that compound **4b** was highly specific toward prostate cancer cells (IC_{50} values were 5 fold higher in RWPE cells), when compared to normal prostate epithelial cells as shown in Figure 3. However, the IC_{50} value for etoposide was 2 fold higher in RWPE cells when compared to PC-3 cells. Similarly, the IC_{50} values for the compounds **4c**, **4e** and **3c** were 1.9, 1.6 and 1.7 fold respectively higher in RWPE cells when compared to PC-3 cells. Hence, the compound **4b** was found be more specific toward cancer cells when compared to etoposide, while the compounds **4c**, **4e** and **3c** have shown almost similar specificity to that of etoposide.

To study the effect of the most potent compound **4b** on cell cycle regulation, PC-3 cells were treated with compound **4b** at concentration of 3, 6 μM for 48 h and incubated with propidium iodide, which was further analyzed by using Muse analyzer. It was observed that there is a marked accumulation of cells in G2/M-phase (1.63 fold) indicating that compound **4b** inhibited the growth of the prostate cancer cells by inhibiting the cell cycle at G2/M-phase as shown in Figure 4.

To check whether the inhibition of proliferation and the resultant cytotoxicity were due to apoptotic induction in the treated cells, morphological features were analyzed by AO/EB (acridine orange/ethidium bromide) staining (Fig. 5). Membrane blebbing, cell shrinkage, apoptotic body formation and chromatin condensation with destructive fragmentation of the nucleus were observed in 3 and 6 μM of **4b** treated cells, whereas these features were absent in the control cells. Quantitation of normal, early apoptotic, late apoptotic, and necrotic stages through AO/EB staining was performed. Whereas control cells exhibited early apoptotic morphology in about 2% of cells, **4b** (1, 3, and 6 μM)-treated cells exhibited the same in 8%, 25% and 42.5% of the cells, respectively.

An in silico study in QikProp 3.7[24] (Schrödinger, 2013) for the tetrahydro-β-carboline-hydantoin hybrids **3a**–**h** and **4a**–**h** were carried out for determining the Lipinski's parameters and the predicted percentage of human oral absorption (% ABS) (Table 2). According to the 'Lipinski's rule of five', the compounds which

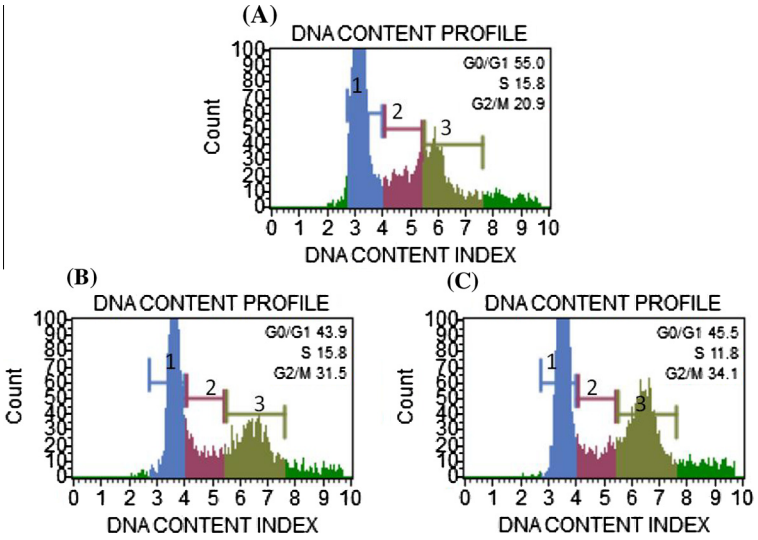


Figure 4. Effect of **4b** on cell cycle progression of prostate cancer cells, (A) PC-3 control cells; (B, C) PC-3 cells treated with 3 and 6 μM for 48 h followed by analysis of cell cycle distribution using propidium iodide cell staining method. All assays were done in duplicate. 1: G0/G1 Phase, 2: S Phase, 3: G2/M Phase.

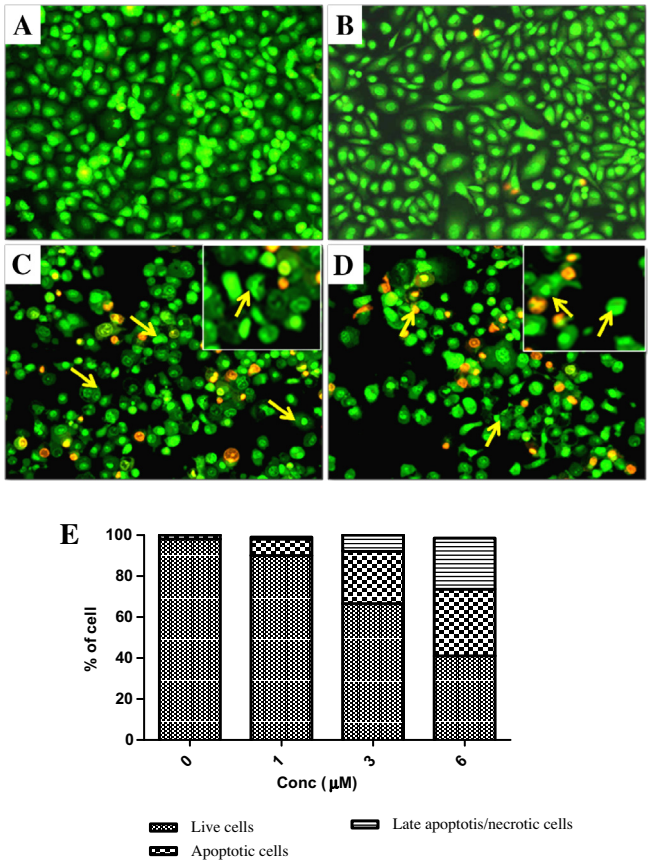


Figure 5. Cellular morphological changes in PC-3 cells treated with and without **4b** for 48 h were investigated by AO/EB staining using fluorescence microscopy. (A) PC-3 control cells showed green live cells with intact membrane and nuclei; (B) cells treated with 1 μM showed live cells with intact membrane and nuclei; (C) cells treated with 3 μM showed early signs of apoptosis characterized by condensed chromatin, cell membrane blebbing, and destructive fragmentation of the nuclei and (D) cells treated with 6 μM showed late apoptotic cells/necrotic cells characterized with orange–red staining of the nucleus after treatment. (E) Percentage of live, apoptotic and necrotic cells at different concentrations of **4b**.

violate more than one of these rules (no. of violations), may face bioavailability issue. It was observed that compounds **3d**, **3h**, **4d**, and **4h** followed the Lipinski's rule of five, indicating more 'drug-like' nature.

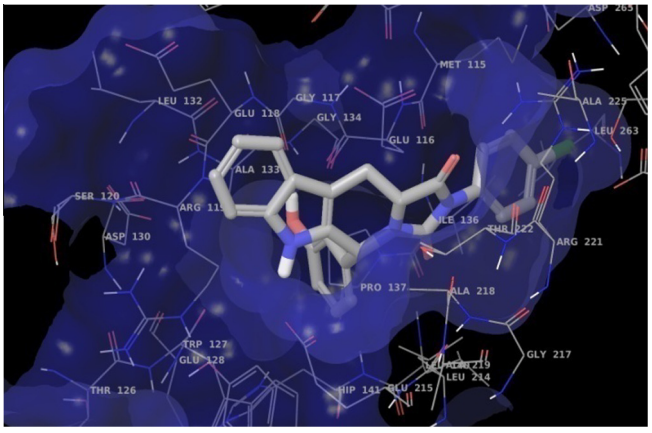


Figure 6. The docked pose of compound **4b** in the binding site of KSP. The compound is depicted with thick rods and the protein is shown with thinner rods. The active site surface is shown in dark blue.

In order to investigate the interaction of the synthesized tetrahydro-β-carboline-hydantoin hybrids **3a–h** and **4a–h** with the Kinesin spindle protein residues, molecular docking was performed using Glide 6.0[25] (Schrödinger, 2013). They were docked into the Kinesin spindle protein obtained from the Brookhaven Protein Data Bank (PDB entry code 1Q0B). Docking results show that all the ligands bound in a site formed from Glu116, Gly117, Glu118, Trp127, Ala133, Ile136, Pro137, Tyr211, Leu214 and Glu215 (Fig. 6). The aromatic ring of phenol group formed π-π stacking with that of Tyr211 and the OH of this phenol group formed H-bond interactions with Gly117 as in the case of HR22C16. The compound **4b** was observed to exhibit the most favourable interactions with the Kinesin spindle protein.

Scatter plot of the Glide scores of the top ranked pose of each compound against the pIC_{50} in PC-3 cell line shows significant correlation with R^2 of 0.826 (Fig. 7).

In conclusion, a series of new hydantoin fused tetrahydro-β-carboline hybrids have been synthesized and evaluated for their in vitro cytotoxicity. These compounds were found to exhibit significant cytotoxicity in the prostate cancer cell line, among which the compound **4b** was found to be the most active. The flow-cytometric analysis studies of compound **4b** in prostate cancer cells indicated G2/M cell cycle arrest. Molecular docking studies indicate effective binding of these compounds with the Eg5 motor

Table 2
Calculated drug-like properties of **3a–h** and **4a–h**[a]

Compound	% ABS	Lipinski's parameters				No. of violations
		*n*HBA (NO)	*n*HBD (OHNH)	log*P*	MW	
3a	100	4	2	5.774	477.442	1
3b	95.09	4	2	5.297	443.888	1
3c	85.28	4	2	5.672	502.366	2
3d	100	4	2	4.376	395.844	0
3e	86.14	5	2	5.887	507.468	2
3f	96.24	5	2	5.422	473.915	1
3g	86.62	5	2	5.871	532.392	2
3h	100	5	2	4.474	425.871	0
4a	95.64	4	2	5.707	477.442	1
4b	92.64	4	2	5.214	443.888	1
4c	83.43	4	2	5.645	502.366	2
4d	100	4	2	4.277	395.844	0
4e	84.63	5	2	5.853	507.468	2
4f	94.58	5	2	5.359	473.915	1
4g	83.83	5	2	5.739	532.392	2
4h	100	5	2	4.401	425.871	0

[a] Number of hydrogen bond acceptor (NO) = *n*HBA; Number hydrogen bond donors (OHNH) = *n*HBD; molecular weight = MW; Partition coefficient = log*P*; Number of violations of Lipinski's rule of five = No. of violations; Predicted percentage of human oral absorption = % ABS.

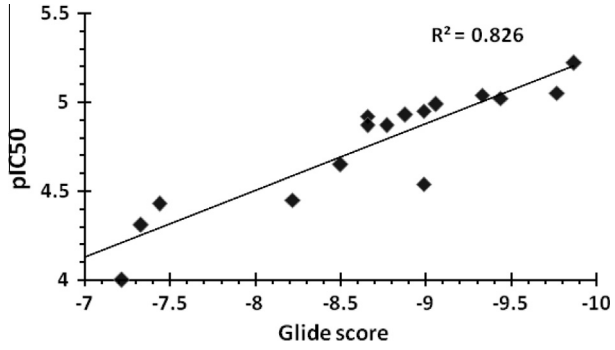


Figure 7. Glide scores plotted versus the pIC_{50} values of compounds **3a**–**h** and **4a**–**h** with the correlation factor R^2.

protein. This group of compounds can be further exploited for obtaining potent cytotoxic compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.038.

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