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Synthesis, cytotoxicity, QSAR, and intercalation study of new diindenopyridine derivatives

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Abstract—Seven new derivatives of diindenopyridine were synthesized by Hantsch pyridine synthesis. Their biological activity to inhibit cell proliferation was assessed by MTT assay on seven cell lines. 11-(4-Fluoro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione and 11-(2-nitro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione were active on K-562 cell line with IC_{50} values of 79.66 and 78.2 μM, respectively. Effect of structural parameters on the cytotoxicity was evaluated by quantitative structure activity relationship (QSAR) analysis and a linear relationship was found between the $-\log IC_{15}$ of these compounds and their surface area and molar refractivity. To model the DNA-intercalator complex, force field molecular mechanic calculation was employed and the binding energy of the reaction between the intercalating agent and each reasonable double base pairs of DNA was calculated. It was found that these molecules could intercalate into the DNA. Also, it was observed that 11-(2-nitro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione, which showed the highest activity in K-562 cell line, produced the most negative binding energy with a moderate selectivity toward A–G/T–C double base pairs.

1. Introduction

The discovery and development of novel therapeutic agents for the treatment of malignancy has a vital importance. The tremendous potential advantages and challenges associated with the use of a molecular approach to cancer drugs have been reviewed.[1] Efforts to understand the basis of antitumor activity of anthracyclines naturally included a focus on the impact of drug into DNA double helix structure. Anthracyclines and DNA intercalators represent one of the most important classes of anticancer drugs in their overall utility in clinical oncology.[2] Doxorubicin, daunorubicin, and amsacrin are now being used for treatment in a wide range of tumors.[2] Camptothecin, isolated from a Chinese tree, is a potent topoisomerase I inhibitor.[3] Ascididemin, a natural pentacyclic aromatic alkaloid has toxicity toward topoisomerase II.[4] The use of 9-hydroxy-2-methyl ellipticinium acetate in clinic has been reported.[5] Derivatives of tetrahydropyrrolo[3,4-*a*]-carbazole-1,3-dione and tetrahydropyrido[3,2-*b*]pyrrolo[3,4-*g*]indole-1,3-dione were screened for cytotoxicity, DNA interaction, and topoisomerase II inhibition potency.[6] Some of the *N*-(alkylamino) alkyl derivatives of pyrimido[5,6,1-*de*]acridine-1,3,7-trione have shown borderline in vivo antitumor activity.[7] 5,11-Dimethyl-5*H*-indole [2,3-*b*] quinolin displayed a strong antibacterial, antimycotic, and cytotoxic activity as well as significant in vivo antitumor properties.[8] New series of 3,5-bis(3′-indolyl)pyrazines and 2,4-bis(3′-indolyl)pyrimidines had a great cytotoxicity against diverse human cell lines.[9] New class of tetracyclic 11-oxo-11H-indeno[1,2-*b*]quinoline-6-carboxamide was reported as potent cytotoxins and potential dual topoisomerase I and II inhibitors.[10] Two common characteristics of these compounds include the presence of a planar polycyclic chromophore, able to intercalate into DNA, and at least one basic side chain, which can increase DNA binding affinity and in some cases increase solubility under physiological conditions. Great efforts have been devoted to modify and determine its optimal

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characteristics. Results indicate that the planar portion of the molecule could be tricyclic, tetracyclic, or even pentacyclic. Although some data indicates a positive relationship between DNA binding and antitumor activity, it is normally accepted that DNA intercalation is a necessary but not sufficient condition for antitumor activity.[11] Also it has been pointed out that DNA binding, while possibly improve intrinsic activity, can be even deleterious to normal distribution and penetration into the cells.[11,12]

Geita and Vang, in 1962, synthesized four derivatives of 11-R-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione in which R were methyl, butyl, isopropyl, and isobutyl.[13] The structure of these compounds are similar to that of intercalating agents, which recently synthesized and evaluated as cytotoxic molecules.[3–10,14] According to these data, we designed and synthesized some new derivatives of 11-R-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione in which R were different substituted phenyl (linear pentacyclic structures) and their cytotoxicity was evaluated by MTT assay.[15]

There are reports of both experimental techniques such as spectrophotometry,[16] spectroflurimetry,[17] NMR,[18] X-ray crystallography,[19] and theoretical techniques such as molecular modeling[20–26] for studying the interaction of intercalators with DNA. Considering the limitations as well as the difficulty of some experimental techniques, molecular modeling, and theoretical organic chemistry have been found as powerful forces in modern organic chemistry.[27] They are widely used and highly respected, but, they have also contributed to some of the erroneous ideas. These methods have been applied to assess the reaction of intercalating agents such as metal complexes,[20,21] indolo[2,3-*b*]quinoline derivatives,[22] Troger bases,[23] quinones,[24] anthramycin derivatives,[25] acrydine orange,[26] and with DNA. In this paper, we investigated the intercalation reaction between the synthesized diindenopyridine molecules and different reasonable double base pairs of DNA by the AMBER force field method. In addition, a quantitative structure activity relationship (QSAR) study [27–32] has been conducted to analyze the effect of various structural parameters of the molecule on its cytotoxic activity.

2. Results and discussion

2.1. Synthesis and antitumor assay

Diindenopyridine analogues (**9a**–**g**) were obtained by photo degradation of related intermediate products, synthesized by classical Hantsch condensation method, in which aryl (alkyl) carboxaldehyde (**2**–**7**) reacted with indandione (**1**) and ammonium acetate (Fig. 1).

The data of cytotoxicity is shown in Table 1. IC_{50} of all of the compounds were more than 100 μM, whereas **9d**

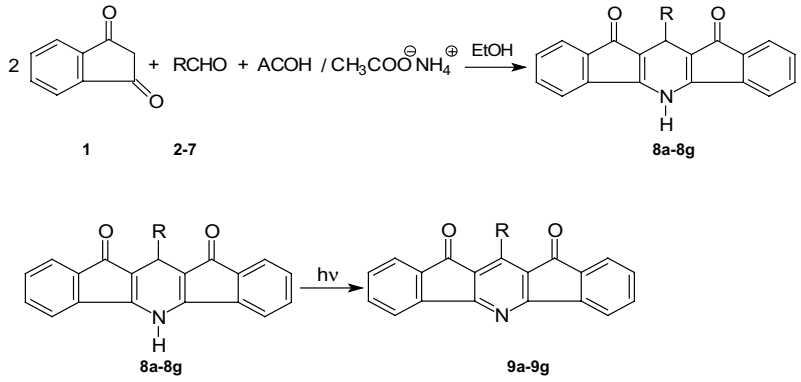

Figure 1. Synthesis of diindenopyridine derivatives.

Table 1. Cell growth inhibitory activity of compounds **9a**–**g** in vitro[a]

Compound	R	IC_{15} (μM)						
		K-562	Fen	Vero	Hela	KB	SK-BR-3	U-937
9a	Methyl	21.4	3.7	>100.0	>100.0	16.0	>100.0	>100.0
9b	Phenyl	17.1	100.0	>100.0	>100.0	2.1	6.7	5.3
9c	3-Fluoro-phenyl	20.0	2.1	43.8	23.4	>100.0	<1.0	>100.0
9d	4-Fluoro-phenyl	3.3	5.1	36.1	2.9	28.6	>100.0	7.5
9e	2-Nitro-phenyl	1.5	35.1	9.0	3.4	17.7	14.3	30.2
9f	4-Nitro-phenyl	3.6	<1	1.3	>100.0	9.5	46.3	>100.0
9g	4-Dimethylamino-phenyl	17.2	4.0	50.3	25.1	>100.0	20.4	60.0
Doxorubicin	—	<1.0	<1.0	1.4	1.3	<1.0	28.3	<1.0
DMSO	—	80.5	>100.0	>100.0	>100.0	>100.0	>100.0	>100.0

[a] IC_{15} is the molar concentration causing 15% growth inhibition of tumor cells. In K-562, IC_{50} of **9d** and **9e** was 79.66 and 78.2 μM, respectively.

and **9e** in K-562 cell line had an IC_{50} equivalent to 79.66 and 78.2 μM, respectively. According to the results, the most cytotoxic effect was seen on K-562 cell line and among the compounds, **9d** and **9e** were more active. Possibly the presence of electrophile moiety at *ortho* or *para* position of the diindenopyridine structure increases it's lethal activity on this cell line.

3D structural representations, obtained by AM1 method, are shown in Figure 2 and some of their electronic features are summarized in the first five rows of Table 2. Angle between the phenyl rings at the ends of the diindenopyridine plane, (α), is around zero so, almost all of the compounds have a flat structure. Meanwhile, the angle between the plane of the C4 substituted phenyl (**9b–g**) and diindenopyridine plane, (β), is about 50° for *ortho* substituted phenyl and about 90° for *para*. The dipole moment (DM) of the molecules are also included in Table 2. Molecule with *para*-nitro substituent (**9f**) was the most polar molecule whereas molecule with *para*-dimethylamino substituent (**9g**) had the least polarity. The polarity of the most active molecule in K-562 (**9e**) is lower than that of **9f** but is greater than the polarity of the others except for **9d**.

2.2. QSAR analysis

For QSAR analysis two types of descriptors were used, which are listed in Table 2. Lipophilicity index ($\log P$), surface area (SA), volume (V), hydration energy (HE), polarizability (Pol), and molar refractivity (MR) are those descriptors, which were calculated from the whole structure of the molecule. The rest are substituent constants parameters including electronic (σ), hydrophobic (π), and STERIMOL parameters (L, B_1, B_5, L/B_1, and B_5/B_1). These parameters are used to account the property of the substituent and are derived for an individual substituent on a molecule. Among the molecules, **9b–g** had an aromatic group on C4 of the pyridine but **9a** contained a methyl group. Therefore, the substituent constant parameters are not shown for **9a** in Table 2.

The QSAR analysis was performed three times. First, all of the molecules with the first type of descriptors were analyzed. The results are shown by Eq. 1:

$$\begin{aligned} &\mathrm{Log1/IC_{15}} = -0.160(\pm 0.040)\mathrm{HE} + 4.272(\pm 0.251),\\ &N = 7, \quad R = 0.850, \quad \mathrm{Se} = 0.231, \quad F = 13.0. \end{aligned} \tag{1}$$

This equation implies that hydration energy of the molecules affected their antitumor activity. The negative sign of the coefficient of the HE proposes that more negative hydration energy are relevant to antitumor activity. As the polar functional groups produce negative hydration energy, it can be concluded that presence of such functional group on the intercalating agents increase their antitumor activity.

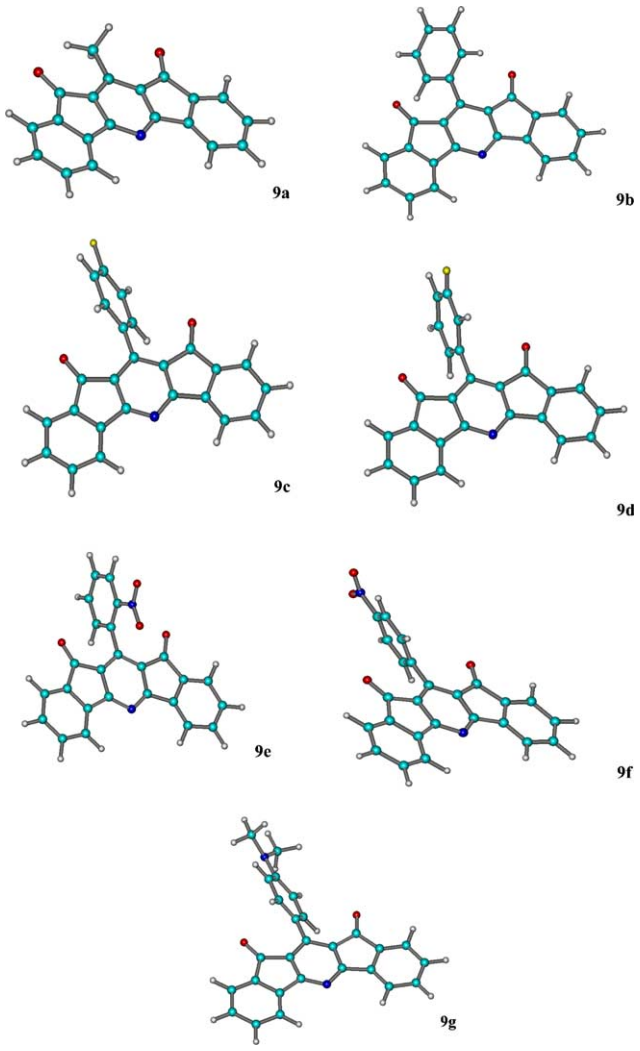

Figure 2. The three dimensional representation of diindenopyridine derivatives.

Second QSAR analysis was performed by using the substituent constant parameters of the molecules **9b–g** and the following equation was obtained:

$$\begin{aligned} &\mathrm{Log1/IC_{15}} = 0.408(\pm 0.084)\sigma + 4.992(\pm 0.080)\\ &N = 6, \quad R = 0.925, \quad \mathrm{Se} = 0.167, \quad F = 23.7 \end{aligned} \tag{2}$$

Among the substituent constants used in this paper, only the Hammet's electronic parameter was used by Eq. 2. This parameter was used to account the electron withdrawing or donating ability of the substituents by Hammet. The positive sign of the coefficient of this parameter describes that by increasing the σ, the cytotoxicity of the compounds under study is increased. Referring to the values of σ in Table 2 reveals that the electronic substituent constant of the electron withdrawing groups such as –F and $-NO_2$ is greater than that of electron donating groups such as $N(CH_3)_2$, and according to the above discussion, the electron withdrawing groups increase the activity of the compounds. Therefore, there is a consistency between the derived QSAR models.

Table 2. The electronic and structural features of diindenopyridine derivatives

Property	9a	9b	9c	9d	9e	9f	9g
E (kcal/mol)	−4172.0	−5091.6	−5101.9	−5103.8	−5264.3	−5268.0	−5798.7
DH_f (kcal/mol)	51.0	90.0	46.5	44.6	97.4	93.7	98.2
DM (D)	3.96	3.54	3.41	5.31	5.26	9.76	1.80
α (°)	0.02	1.00	0.03	−1.15	−0.10	−1.04	−0.10
β (°)	—	53.66	90.00	52.72	91.07	54.84	50.60
SA (Å^2)	484.14	568.90	570.13	561.74	587.31	586.60	633.35
V (Å^3)	822.03	976.14	974.65	976.17	1014.48	1017.25	1102.71
HE (kcal/mol)	−3.30	−4.70	−4.69	−4.59	−7.29	−9.17	−3.23
log P	3.72	4.94	5.08	5.08	4.89	4.89	5.20
Pol	33.17	41.00	40.91	40.95	42.84	42.88	46.02
MR	87.50	107.60	107.82	107.82	114.93	114.93	122.03
σ	—	−0.01	0.34	0.06	1.49	0.78	−0.83
π	—	1.96	0.14	0.14	−0.28	−0.28	0.18
L	—	6.28	2.65	2.65	3.44	3.44	3.53
B_1	—	1.71	1.35	1.35	1.70	1.70	1.35
B_5	—	3.11	1.35	1.35	2.44	2.44	3.08
B_5/B_1	—	1.82	1	1	1.44	1.44	2.28
L/B_1	—	3.67	1.96	1.96	2.02	2.02	2.62

Attempts were also made to use all of the derived descriptors to perform third QSAR analysis of the **9b–g**. The resulted models were those that obtained previously (Eqs. 1 and 2) and no new one was obtained. Comparison of the observed relationships from Eqs. 1 and 2 reveal that hydrophile molecules with electron withdrawing substituent produce higher cytotoxicity in comparison to other molecules. It should be noted that the statistical quality of the resulted equation is not high and therefore they can not be used for the accurate prediction of the activity. However, according to the discussion made, they could explain the structure activity relationships, well.

2.3. Intercalation study

We adopted molecular mechanic and after that semi empirical quantum chemical method to dock the intercalators into every reasonable double base pairs of DNA (A–T/A–T, A–T/T–A, A–T/G–C, A–T/C–G, C–G/G–C, C–G/C–G). The double base pairs of DNA were built by the nucleic acid database of Hyperchem and their 3D geometry were optimized by AM1 semi empirical method (Fig. 1). The binding energy and ΔH_f were calculated for each double base pairs. In molecular modeling studies, finding a suitable force field is important. Some of the force field methods did not result in a reasonable 3D structure for our work but the AMBER did well. The results showed that these molecules are able to intercalate into the DNA. After finding the optimum geometry for the intercalation complexes by the AMBER force field method, the AM1 semi empirical method was used to calculate the binding energy (E) and ΔH_f of the resulted complexes. Enthalpy difference (ΔH) and binding energy difference (ΔE) of the intercalation complex was calculated by subtraction of the quantity of the complex from the sum of the quantities of compounds and DNA [$\Delta E = E_{complex} - (E_{compound} + E_{DNA})$ and $\Delta H = \Delta H_{f\ complex} - (\Delta H_{f\ compound} + \Delta H_{f\ DNA})$].[33] The results are represented in Tables 3 and 4. Molecule **9e**, with the highest activity in K-562, produced the strongest complex with DNA relative to the others. This was measured by the smallest ΔH and ΔE values of intercalation. By comparison of the results obtained from different DNA double base pairs, it can be concluded that the compound **9e** has a moderate selectivity toward the A–T/G–C double base pairs.

In an attempt to find a relationship between the ΔH or ΔE of intercalation and antitumor activity, the $-\log IC_{15}$ was plotted against different columns of Tables 3 and 4. The correlation coefficient obtained for each double

Table 3. ΔE (kcal/mol) of the intercalation reaction between the studied molecules and different double base pairs of DNA

Molecule	DNA double base pairs					
	A–T/T–A	A–T/C–G	A–T/A–T	A–T/G–C	C–G/G–C	G–G/C–C
9a	−6.6	−21.4	−19.1	−13.7	−17.9	−15.1
9b	−19.7	−23.4	−42.2	−15.8	−30.7	−11.4
9c	−15.9	−10.8	−1.5	−14.7	−22.7	−1.5
9d	−49.9	−41.3	−24.6	−44.1	−50.6	−32.3
9e	−42.4	−35.4	−11.5	−53.4	−37.2	−25.6
9f	−32.4	−41.4	−16.8	−44.2	−31.0	−25.6
9g	−24.6	−21.6	−47.6	−12.3	−21.1	−10.2
$R^{2\,a}$	0.511	0.656	0.230	0.928	0.364	0.685

[a] R^2 is the square of the correlation coefficient for the plot of activity against the ΔE of intercalation.

Table 4. ΔH (kcal/mol) of the intercalation reaction between the studied molecules and different double base pairs of DNA

Molecule	DNA double base pairs					
	A–T/T–A	A–T/C–G	A–T/A–T	A–T/G–C	C–G/G–C	G–G/C–C
9a	−13.5	−18.2	−29.7	−22.7	−15.9	−8.9
9b	−19.1	−32.4	−12.6	−22.4	−36.1	−8.7
9c	−12.8	−17.5	−5.3	−12.4	−25.9	−1.0
9d	−44.6	−47.3	−45.5	−48.2	−53.3	−42.9
9e	−33.9	−43.4	−35.8	−68.1	−45.9	−45.2
9f	−38.0	−52.3	−23.2	−49.9	−34.0	−23.1
9g	−16.3	−28.7	−18.9	−12.3	−29.6	−3.6
$R^{2\,a}$	0.654	0.564	0.481	0.934	0.331	0.792

[a] R^2 is the square of the correlation coefficient for the plot of activity against the ΔH of intercalation.

base pairs is represented in the last row of Tables 3 and 4. It shows that the ΔH and ΔE of the reaction between intercalators and A–T/G–C double base pairs have more correlation with the $-\log IC_{15}$ relative to those obtained from other double base pairs. These observation reveal that the A–T/G–C has a significant role in interaction between the studied molecules and DNA.

The 3D structure of the intercalation complexes formed between the molecule **9e** and different double base pairs of DNA (except for A–T/G–C) are shown in Figure 3 and that of A–T/G–C in three different side views are shown in Figure 4. As we see, the molecule **9e** is more or less intercalated into the DNA. However this molecule penetrates into the A–T/G–C double base pairs more than the others. When **9e** intercalates into A–T/G–C, the hydrogen bond between the guanine and cytosine is broken and the oxygens of the nitro group of **9e** form new hydrogen bonds with hydrogens of the guanine.

3. Experimental

3.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H NMR spectra were run at a Varian Unity Plus 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) relative to TMS as an internal standard. The mass spectra were measured with a HP 6890 spectrometer at 70 eV. The IR spectra were obtained by using a Perkin Elmer Paragon 1000 spectrophotometer (KBr disks). Microanalyses were within ±0.40% of theoretical values for C, H, and N. All spectra were consistent with the

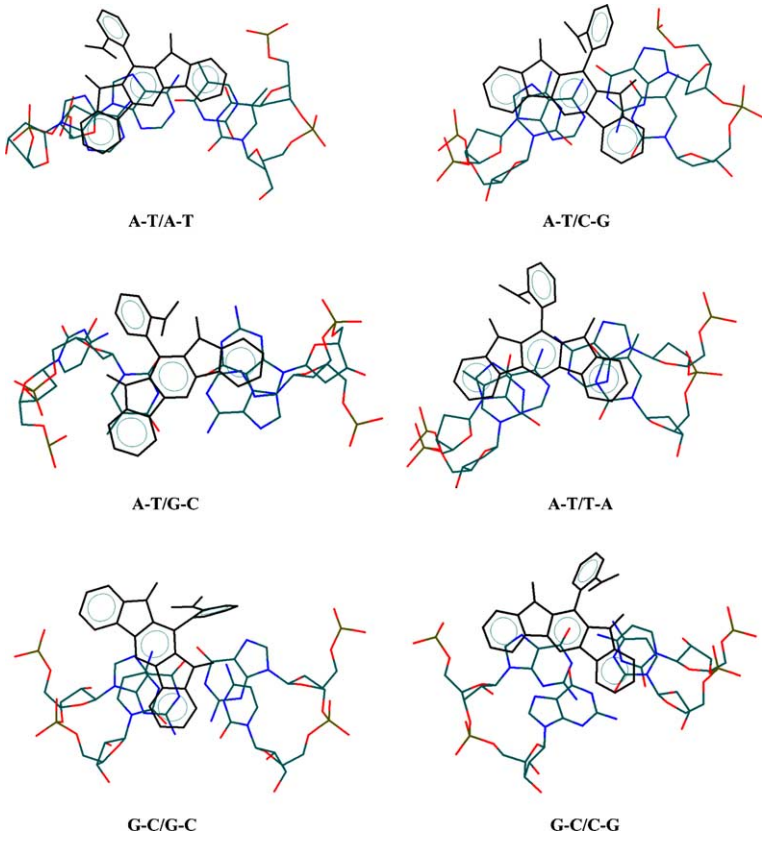

Figure 3. Optimized structure of the intercalation complex of **9e** with different DNA double base pairs.

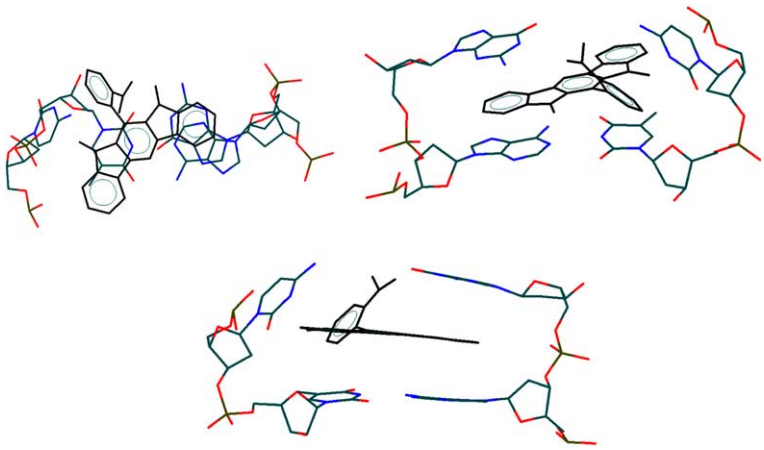

Figure 4. Optimized structure of **9e**-(A–T/G–C) intercalation complex in three different views.

assigned structures. All reagents were purchased from the Aldrich Chemical Co.

3.1.1. General procedure for synthesis. Ammonium acetate (1.54 g, 20 mmol) was added to a stirring solution of indandione **1** (1.46 g, 10 mmol) and aryl(alkyl)carboxaldehydes **2**–**7**, (5 mmol) in 50 mL of absolute acetic acid and pure ethanol (1:3). The reaction was refluxed at 80 °C for 3 h under exposure of a 100 W tungsten lamp. After that the reaction mixture was cooled, the precipitated sticky solid was removed by filtration. Then it was washed with pure ethanol and purified by silica gel thin layer chromatography using chloroform.

3.1.2. 11-Methyl-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9a). Yield 8%. ^{1}H NMR ($CDCl_3$): δ 7.65 (m, 8H, diindeno), 2.97 (s, 3H, CH_3). IR (KBr): ν 3019 (CH, aromatic), 1712 cm^{-1} (C=O, ketone). MS (m/z rel intensity): 297 (M^+, 100), 269 (6), 240 (22).

3.1.3. 11-Phenyl-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9b). Yield 21.7%. IR (KBr): ν 3020 (CH, aromatic), 1716 cm^{-1} (C=O, ketone). MS (m/z rel intensity): 359 (M^+, 100), 301 (12), 179 (14), 151 (11).

3.1.4. 11-(3-Fluoro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9c). Yield 9.97%. IR (KBr): ν 3018 (CH, aromatic), 1713 cm^{-1} (C=O, ketone). MS (m/z rel intensity): 377 (M^+, 100), 376 (69), 323 (13), 188 (10).

3.1.5. 11-(4-Fluoro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9d). Yield 16.9%. IR (KBr): ν 3021 (CH, aromatic), 1714 cm^{-1} (C=O, ketone). MS (m/z rel intensity): 377 (M^+, 100), 348 (9), 323 (12), 188 (8).

3.1.6. 11-(2-Nitro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9e). Yield 2.9%. IR (KBr): ν 3019 (CH, aromatic), 1712 (C=O, ketone), 1530 and 1344 cm^{-1} (NO_2). MS (m/z rel intensity): 404 (M^+, 100), 372 (95), 358 (80), 303 (21).

3.1.7. 11-(4-Nitro-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9f). Yield 6.38%. IR (KBr): ν 3022 (CH, aromatic), 1708 (C=O, ketone), 1536 and 1353 cm^{-1} (NO_2). MS (m/z rel intensity): 404 (M^+, 100), 374 (8), 358 (48), 301 (20).

3.1.8. 11-(4-Dimethylamino-phenyl)-diindeno[1,2-*b*;2′,1′-*e*]pyridine-10,12-dione (9g). Yield 15.9%. ^{1}H NMR ($CDCl_3$): δ 7.81 (m, 8H, diindeno), 7.62 (dd, 4H, phenyl), 3.13 (s, 6H, $N(CH_3)_2$). IR (KBr): ν 3019 (CH, aromatic), 1708 cm^{-1} (C=O, ketone). MS (m/z rel intensity): 402 (M^+, 100), 401 (27), 348 (23), 326 (14), 277 (37).

3.2. Cell culture

Seven cell lines including Vero (monkey kidney primary cells), Hela (human cervix carcinoma), K-562 (human chronic myeloid leukemia), SK-BR-3 (human breast adenocarcinoma), Fen (human bladder epithelial carcinoma), U-937 (human histiocytic lymphoma) and KB (human cervix carcinoma, a derivative of Hela) were all obtained from the Iran Pasteur Institute, Tehran, Iran, except for the Fen cell line, which was kindly provided by Dr. A. M. E. Nouri, Department of Medical Oncology, The Royal London Hospital Trust, London, UK. Cells were cultured in RPMI-1640 (Sigma, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 IU/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in 50 cm^3 flask (Nunc, Denmark) with 5 mL of culture medium in a humified 5% CO_2 incubator at 37 °C.

3.3. Cytotoxicity evaluation

Appropriate amount from each one of the compounds was mixed with DMSO (dimethyl sulfoxide). Then with

serial dilution in RPMI-1640 four concentrations (10, 100, 500, 1000 μM) were made. DMSO, as negative control, diluted with the same method. For positive control, doxorubicin was mixed with PBS (phosphated buffered saline) to make the similar concentrations. MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide], (Sigma, USA), was dissolved in PBS at 5 mg/mL and then filtered to remove any undissolved particle. Each well of the microtiter plate was filled with $1–5\times10^4$ cells (depending on the cell line) in 90 μL culture medium. Then, 10 μL from the stock solutions of the compounds, negative and positive controls was added as triplicate to the wells to reach the final concentration of 1, 10, 50, and 100 μM. Three wells containing only the same number of the cells left in each plate. Plates were kept in a humified incubator for 48 h. After the incubation period, MTT assay was carried out by the procedure described by Jabbar et al.[15]

3.4. Statistical analysis

Data were analyzed using SPSS software; one way ANOVA and Duncan test. A confidence level of $\leqslant 0.05$ was considered significant.

3.5. QSAR and molecular modeling

All molecular modelings was carried out using Hyperchem software (ver. 7). The 3D geometry of the compounds was optimized by the AM1 Hamiltonian. Molecular descriptors were computed by the Hyperchem and the substituent constants were obtained from the literature. The MLR analysis was performed by the SPSS software using the stepwise selection and elimination procedure for variable selection.

For intercalation studies, double base pairs of DNA were built by nucleic acid database of the Hyperchem software and 3D geometry was optimized by AM1 method. For the determination of the structures of the DNA-intercalator complexes, the procedure of the Xiong and Yang was used.[20] The intercalators were brought close to the DNA double base pairs so that the plane of diindenopyridine became parallel to the planes of the nucleic acids. Then, the intercalators were allowed to slide into the double base pairs of DNA using geometry minimization procedure of Hyperchem software. Energy minimization was performed using the steepest-descent minimization algorithm in vacuum, making use of the AMBER force field. Energy minimizations were continued until the derivatives (RMS) were less than or equal to 0.01 kcal/mol Å.

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References and notes

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