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RESEARCH ARTICLE

Synthesis and antiproliferative activity evaluation of imidazole-based indeno[1,2-b]quinoline-9,11-dione derivatives

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Abstract A series of new imidazole substituted indeno [1,2-b]quinoline-9,11-dione derivatives were synthesized and evaluated for their antiproliferative effects on HeLa, LS180, MCF-7 and Jurkat human cancer cell lines. Antiproliferative effects were evaluated using MTT assay. Prepared compounds exhibited weak to good antiproliferative activity in evaluated cell lines. Prepared compounds were more potent in Jurkat cell line when compared to LS180, HeLa and MCF-7 cell lines. Compounds **29** ($IC_{16} = 0.7$ μM) and **31** ($IC_{16} = 1.7$ μM) and **33** ($IC_{16} = 1.7$ μM) were found to be the most potent molecules on Jurkat cell lines. Moreover; it was found that some of the tested compounds bearing imidazole-2-yl moiety on the C_{11}-position of dihydropyridine ring exhibited superior antiproliferative activity in comparison to *cis*-platin especially in Jurkat cell line (compounds **29**, **31**, and **33**). It seemed that the introduction of electron-withdrawing groups on the imidazole ring enhanced the antiproliferative potential of these compounds (compounds **27**, **29** and **31**). The results of this study proposed that some of the imidazole substituted indeno[1,2-b]quinoline-9,11-dione compounds may act as efficient anticancer agents in vitro, emphasizing their potential role as a source for rational design of potent antiproliferative agents.

Keywords Synthesis · Indeno[1,2-b]quinoline-9,11-dione · Antiproliferative · MTT assay

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Introduction

Dihydropyridines (DHPs) are heterocyclic structures that have found significant attention among researchers. Many efforts on ruling synthetic routes for DHP scaffolds began after the first successful synthesis of symmetric 1,4-DHP derivative via one-pot cyclocondensation reaction of ammonia with alkylacetoacetate and aldehyde more than a century ago (Hantzsch 1882). Since then, a variety of synthetic procedures have been elucidated for preparation of symmetrical and unsymmetrical DHP derivatives (Vanden Eynde and Mayence 2003; Saini et al. 2008; Swarnalatha et al. 2011; Hugel 2009).

Besides synthetic feature, DHPs have been looked upon as one of the most important medicinal scaffolds possessing interesting biological properties such as anti-hypertensive (Nekooeian et al. 2010; Shafiee et al. 2002), calcium channel modulating (Miri et al. 2008; Davood et al. 2006), antituberculosis (Shafii et al. 2008), anticoagulant (Kumar et al. 2011), antidyslipidemic (Kumar et al. 2010), anti-oxidant (Kumar et al. 2010; Leon et al. 2008), and multidrug resistance reversal (MDR) (Tasaka et al. 2001) activities.

In this regard, several reports indicating anti-tumor activity of symmetric 3,5-dicarboxamide (Sirisha et al. 2010), 3,5-diketo (Engi et al. 2006; Bazargan et al. 2008), 3,5-dicarboxylate (Engi et al. 2006; Foroughinia et al. 2008) and 3,5-dicyano (Abbas et al. 2010) derivatives of

1,4-DHPs have been documented in the literature. Dexniguldipine is a prototype of the unsymmetrical 1, 4-DHPs exhibiting therapeutic activity in neuroendocrine hamster lung tumors (Hahn et al. 1997). Rigid polycyclic scaffolds constituted around a DHP core for which 1,8-acridinediones are representative ones have also been reported to possess anti-tumor activity (Jamalian et al. 2011). One possible rationalization toward the observed antiproliferative activity of rigid polycyclic compounds is their planar polycyclic portion which enables them to intercalate properly with DNA molecule (Miri et al. 2012; Brana et al. 2001; Ryabokon et al. 2005).

Some in vitro assays revealed that 3,5-diketo derivatives of 1,4-DHPs could decrease DNA damage and motivate its repair in Raji, HL60 and peripheral blood lymphocyte cells. Jamalian and colleagues have demonstrated that potential DNA-intercalating activity of 1,8-acridinones could be achieved via incorporating imidazole moiety bearing electron-withdrawing groups (Jamalian et al. 2011). Moreover there are some reports highlighting the effect of DHPs on potentiation of anti-tumor activity of general cytotoxic drugs (Fedeli et al. 1989). For instance, structural conjugation of homocamptothecins with DHP scaffold has proven to reinforce the inhibition of DNA topoisomerase I (Zhu et al. 2011). Such enhanced activities have also been reported for telupidine, a DHP derivative that was superior to veapamil in enhancing the anti-tumoral activities of anthracyclines and vinca alkaloids in vitro (Tolomeo et al. 1994). It is worth noting here that MDR reversing activity of 1,4-DHPs especially those containing aromatic rings may be of significant importance in potentiating the cytotoxic/antiproliferative action of anti-cancer agents (Kiue et al. 1990).

In continuation to our work on novel fused and condensed 1,4-DHP structures (Miri et al. 2004, 2010), we report here the synthesis and characterization of new indeno[1,2-b]quinoline-9,11-dione derivatives. Furthermore all the prepared compounds were subjected to in vitro antiproliferative assay on four different human cancer cell lines.

Materials and methods

Chemistry

Synthesis of desired Indeno[1,2-b]quinoline-9,11-diones was achieved following steps depicted in Figs. 1 and 2. Indeno[1,2-b]quinoline-9,11-dione derivatives **19–31** were achieved by molecular condensation of equivalent amounts of corresponding aldehydes (**7–11**), enamines (**14–17**) and 1,3-indandione (**18**). At the next stage, oxidative aromatization of the compounds **19–31** by MnO_2 afforded the corresponding aromatized indeno[1,2-b]quinoline-9,11-diones (**32–40**). The yield and some physicochemical characteristics of final products are summarized in Tables 1 and 2.

Melting points were determined with a Reichert–Jung hot-stage microscope and were uncorrected. All the synthesized compounds were characterized by mass spectroscopy, IR and ^{1}H NMR. IR spectra were recorded on a Nicolet FT-IR Magna 550 spectrophotometer. ^{1}H NMR spectra were determined by a Bruker FT-500 MHz

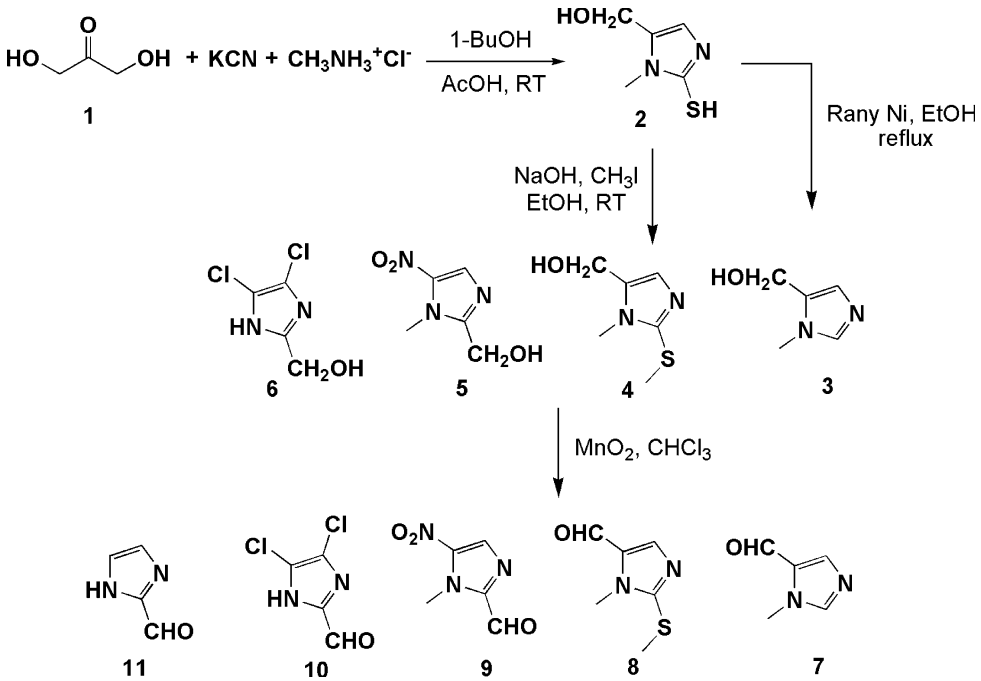


Fig. 1 General structure and synthetic procedure for preparation of aldehydes used in the synthesis of indeno[1,2-b]quinoline-9,11-dione

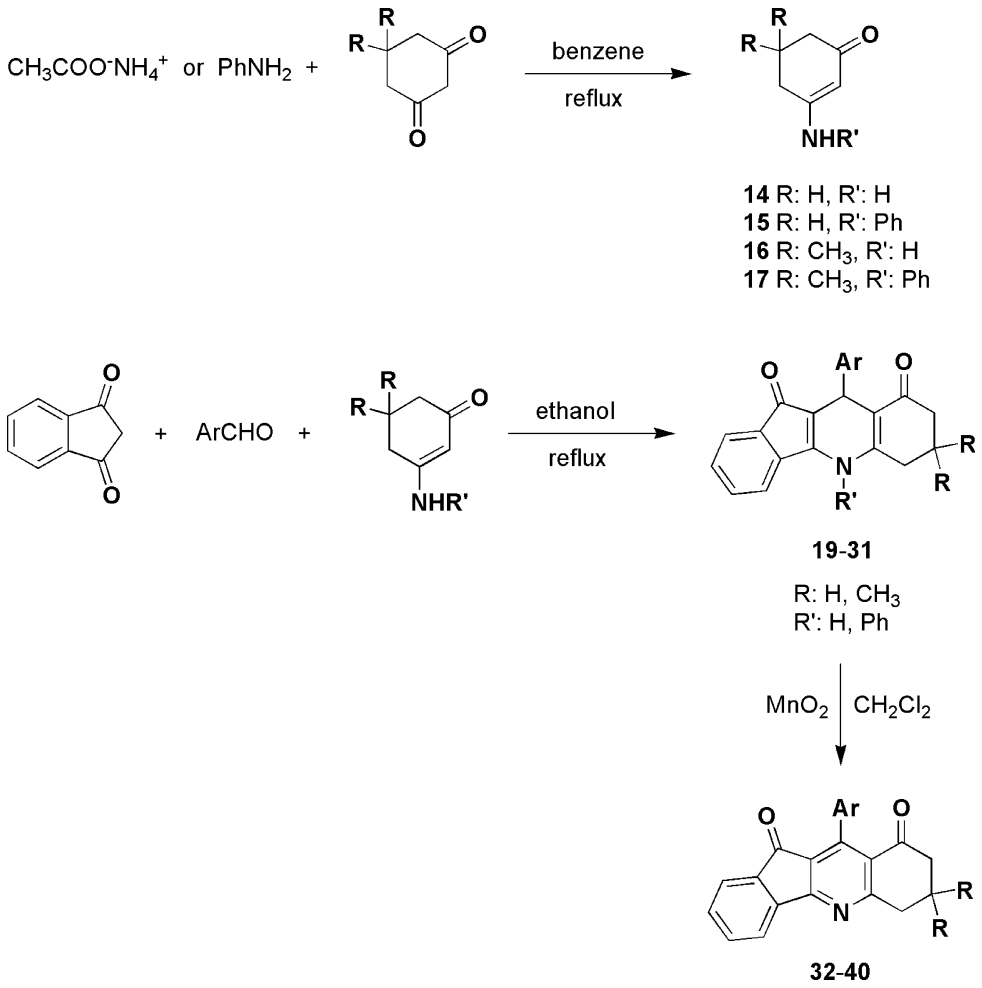


Fig. 2 General synthetic route to indeno[1,2-b]quinoline-9,11-diones

spectrometer in chloroform-d_1 or DMSO-d_6. All the chemical shifts were reported as δ values (ppm) against tetramethylsilane as an internal standard. The MS spectra were recorded using a Finnigan TSQ-70 spectrometer at 70 eV.

General procedure for the synthesis of imidazolyl aldehydes, 7–11

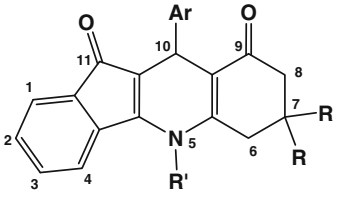
1H-imidazole-2-carbaldehyde (**11**) was obtained from a commercial source and all other required aldehydes were prepared via oxidation of corresponding alcohols (**7**–**10**). For this purpose, MnO_2 (10 equiv.) was added to a stirred solution of corresponding alcohols in chloroform (1 equiv.) The mixture was refluxed overnight and then filtered. The filterate was dried and recrystallized from methanol to give corresponding aldehydes (70–80 % yields) (**7**–**10**) (Javidnia et al. 2011).

(2-Mercapto-1-methyl-1H-imidazol-5-yl) methanol (**2**) used for synthesis of **7** and **8**, was obtained via stirring a mixture of dihydroxyacetone (**1**), potassium thiocyanate and methylamine hydrochloride in acetic acid/1-butanol at room temperature (m.p. 203–205 °C, 55 % yield) (Fassihi et al. 2004). (1-methyl-1H-imidazol-5-yl) methanol (**3**) was obtained from hydrogenolysis of compound **2** via refluxing in the presence of Raney nickel in the ethanol (m.p. 115–118 °C, 93 % yield) (Amini et al. 2002). (1-methyl-2-(methylthio)-1H-imidazol-5-yl) methanol (**4**) was obtained through deprotonation using sodium hydroxide solution, and then stirring with methyl iodide in ethanol at room temperature (m.p. 81–90 °C, 17 % yield) (Amini et al. 2002).

General procedure for the synthesis of 3-aminocyclohex-2-enones, 14–17

3-Aminocyclohex-2-enones (**14**, **15**) were prepared via reaction of ammonium acetate or aniline with 1,3-cyclohexanedione (**12**) in benzene using a dean-stark trap apparatus [**14**: 44 % yield and m.p. 103–105 °C, MS: m/z (%) 111 (M^+, 62), 84 (100), 68 (21), 56 (40); **15**: 27 % yield and m.p. 170–173 °C, MS: m/z (%) 187 (M^+, 41), 159 (92), 130 (100), 92 (28), 77 (26)].

Table 1 Chemical structures and physical properties of indeno[1,2-b]quinoline-9,11-dione derivatives



19-31

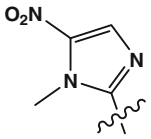
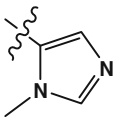
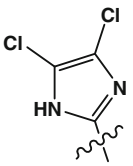
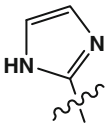
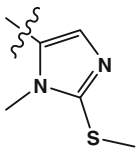
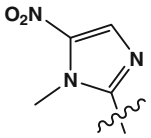
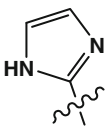
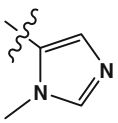
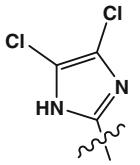
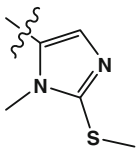
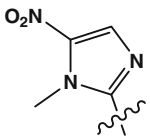
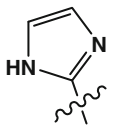
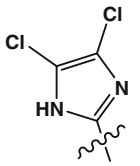
Comp. No.	MW	Ar	R	R′	Mp (°C)	Yield (%)
19	376		H	H	282–285	16
20	331		H	H	280 (decomposition)	38
21	386		H	H	303–305	57
22	393		H	Ph	244–248	46
23	453		H	Ph	241–245	63
24	404		CH_3	H	300–303	43
25	345		CH_3	H	264–265	30
26	359		CH_3	H	257–260	53

Table 1 continued

Comp. No.	MW	Ar	R	R′	Mp (°C)	Yield (%)
27	414		CH_3	H	281–285	49
28	405		CH_3	H	257–260	87
29	480		CH_3	H	309–312	67
30	421		CH_3	Ph	251–255	56
31	490		CH_3	Ph	255–257	69

3-Amino-5,5-dimethylcyclohex-2-enones (**16**, **17**) were prepared via reaction of ammonium acetate or aniline with 5,5-dimethyl-cyclohexanedione (**13**) through a solvent free procedure in oil bath [**16**: 91 % yield and m.p. 161–163 °C, MS: m/z (%) 139 (M^+, 22), 83 (100), 55 (50); **17**: 95 % yield and m.p. 175–185 °C, MS: m/z (%) 215 (M^+, 71), 197 (29), 156 (100), 130 (15)].

General procedure for the synthesis of indeno[1,2-b]quinoline-9,11-diones, **19–23**

As it is illustrated in Fig. 2, all indeno[1,2-b]quinoline-9,11-dione derivatives (**19–23**) were synthesized using the modified Hantzsch classical condensation procedure. After stirring mixture of aldehyde (1 equiv.), corresponding enamine (1 equiv.) (**14**, **15**) and 1,3-indanedione (**18**) in ethanol for 24 h, the mixture was cooled in ambient temperature, concentrated under reduced pressure and further purified by column chromatography with the ethyl acetate/petroleum ether as eluent to afford compounds **19–31** in fair to good yields (16–87 %).

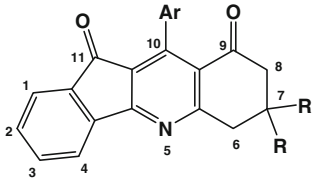
*10-(1-Methyl-5-nitro-1H-imidazol-2-yl)-7,8-dihydro-5H-indeno[3,2-b]quinoline-9,11(6H,10H)-dione (**19**) ($C_{20}H_{16}N_4O_4$)*

^{1}H-NMR ($CDCl_3$): δ 2.01 (m, 2H, H_7), 2.32 (m, 2H, H_6), 2.73 (t, 2H, H_8), 4.19 (s, 3H, N–CH_3), 4.93 (s, 1H, H_{10}), 7.27–7.61 (m, 4H, H_{1-4}), 7.91 (s, 1H, H_4-imidazole), 10.69 (brs, 1H, NH); MS: m/z (%) 376 (M^+, 43), 359 (27), 329 (8), 275 (22), 250 (100), 194 (21), 164 (20), 69 (14), 55 (36); IR (KBr) ν (cm^{-1}): 3503, 2930, 2853, 1680, 1649, 1511, 1368. Anal. Calcd. for $C_{20}H_{16}N_4O_4$: C, 63.82; H, 4.28; N, 14.89; Found: C, 63.95; H, 4.11; N, 14.66.

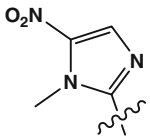
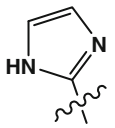
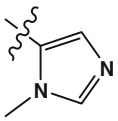
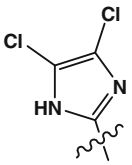
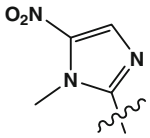
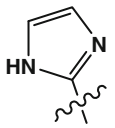
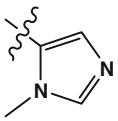
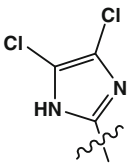
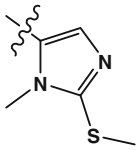
*10-(1-Methyl-1H-imidazol-5-yl)-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**20**) ($C_{20}H_{17}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.97 (m, 2H, H_7), 2.29 (t, 2H, H_6), 2.71 (t, 2H, H_8), 3.82 (s, 3H, N–CH_3), 4.65 (s, 1H, H_{10}), 6.45 (s, 1H, H_4-imidazole), 7.33–7.58 (m, 5H, H_{1-4} and H_2 imidazole), 10.54 (brs, 1H, NH); MS: m/z (%) 331 (M^+,

Table 2 Chemical structures of the aromatized indeno[1,2-b]quinoline-9,11-dione derivatives



32-40

Comp. No.	MW	Ar	R	Mp (°C)	Yield (%)
32	374		H	245–248	54
33	315		H	240–243	16
34	329		H	251–254	62
35	384		H	272–276	56
36	402		CH_3	221–225	70
37	343		CH_3	271–275	46
38	357		CH_3	211–215	45
39	412		CH_3	>300 (decomposition)	56
40	403		CH_3	183–187	68

100), 274 (75), 250 (66), 194 (37), 166 (53), 139 (45), 102 (38), 83 (65), 55 (13); IR (KBr) ν (cm^{-1}): 3442, 3155, 3114, 2935, 1685, 1644, 1511, 1342. Anal. Calcd. for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68; Found: C, 72.23; H, 5.38; N, 12.89.

*10-(4,5-Dichloro-1H-imidazol-2-yl)-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**21**) ($C_{19}H_{13}Cl_2N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.96 (m, 2H, H_7), 2.30 (t, 2H, H_6), 2.70 (t, 2H, H_8), 4.71 (s, 1H, H_{10}), 7.29–7.59 (m, 4H, H_{1-4}), 10.58 (brs, 1H, NH), 13.15 (brs, 1H, imidazole NH); MS: m/z (%) 389 [$(M+4)^+$, 6)], 387 [$(M+2)^+$, 39)], 385 (M^+, 60), 357 (16), 327 (100), 295 (17), 248 (64), 193 (38), 163 (66), 136 (45), 102 (53), 62 (88); IR (KBr) ν (cm^{-1}): 3447, 3140, 2919, 1685, 1614, 1501, 1363, 707. Anal. Calcd. for $C_{19}H_{13}Cl_2N_3O_2$: C, 59.08; H, 3.39; N, 10.88; Found: C, 59.32; H, 3.61; N, 10.58.

*10-(1H-imidazol-2-yl)-5-phenyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**22**) ($C_{25}H_{19}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.25 (m, 2H, H_7), 2.15 (m, 2H, H_6), 2.38 (t, 2H, H_8), 5.01 (s, 1H, H_{10}), 5.14 (2 s, 2H, H_4 and H_5-imidazole), 6.98–7.59 (m, 4H, H_{1-4}), 7.68–7.69 (m, 5H, N-phenyl), 11.64 (brs, 1H, imidazole NH); MS: m/z (%) 393 (M^+, 95), 336 (31), 325 (53), 315 (100), 287 (31), 240 (22), 190 (17), 152 (18), 91 (19), 76 (88); IR (KBr) ν (cm^{-1}): 3063, 2884, 1680, 1634, 1393. Anal. Calcd. for $C_{25}H_{19}N_3O_2$: C, 76.32; H, 4.87; N, 10.68; Found: C, 76.56; H, 4.68; N, 10.39.

*10-(1-Methyl-2-(methylthio)-1H-imidazol-5-yl)-5-phenyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**23**) ($C_{27}H_{23}N_3O_2S$)*

^{1}H-NMR ($CDCl_3$): δ 1.74 (m, 2H, H_7), 1.96 (m, 2H, H_6), 2.33 (m, 2H, H_8), 2.50 (s, 3H, S-CH_3), 3.98 (s, 3H, N-CH_3), 4.94 (s, 1H, H_{10}), 6.75–7.45 (m, 5H, N-phenyl), 7.58–7.65 (m, 4H, H_{1-4}); MS: m/z (%) 453 (M^+, 100), 407 (14), 376 (36), 325 (35), 271 (15), 198 (7), 77 (9); IR (KBr) ν (cm^{-1}): 3058, 2919, 1685, 1634, 1363, 692, 758. Anal. Calcd. for $C_{27}H_{23}N_3O_2S$: C, 71.50; H, 5.11; N, 9.26; Found: C, 71.76; H, 5.34; N, 9.19.

General procedure for the synthesis of indeno[1,2-b]quinoline-9,11-diones, **24–31**

As it is illustrated in Fig. 2, all indeno[1,2-b]quinoline-9,11-dione derivatives (**24–31**) were synthesized using the

modified Hantzsch classical condensation procedure. A mixture of aldehyde (1 equiv.), corresponding enamine (1 equiv.) (**16**, **17**) and 1,3-indanedione (**18**) was refluxed in oil bath (120 °C) and termination of the reaction was confirmed by TLC after 3–5 h. The obtained mixture was cooled at room temperature and poured into cold water. The precipitated solid was filtered and washed with water and ethanol. After dryness, recrystallization from ethanol produced the desired products **24**–**31** in fair to good yields.

*7,7-Dimethyl-10-(1-methyl-5-nitro-1H-imidazol-2-yl)-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**24**) ($C_{22}H_{20}N_4O_4$)*

^{1}H-NMR ($CDCl_3$): δ 1.04 and 1.08 (2 s, 6H, gem-dimethyl), 2.09 and 2.83 (2d, 2H, J = 16 H_6), 2.55 and 2.66 (2d, 2H, J = 17 Hz, H_8), 4.19 (s, 3H, N–CH_3), 4.93 (s, 1H, H_{10}), 7.29–7.60 (m, 4H, $H_{1\text{-}4}$), 7.91 (s, 1H, H_4-imidazole), 10.64 (brs, 1H, NH); MS: m/z (%) 404 (M^+, 64), 387 (79), 317 (22), 278 (100), 231 (14), 222 (30), 193 (36), 138 (13), 80 (15), 55 (29); IR (KBr) ν (cm^{-1}): 3442, 3150, 3026, 2925, 2838, 1685, 1650, 1516, 1378. Anal. Calcd. for $C_{22}H_{20}N_4O_4$: C, 65.34; H, 4.98; N, 13.85; Found: C, 65.06; H, 5.16; N, 13.57.

*10-(1H-imidazol-2-yl)-7,7-dimethyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**25**) ($C_{21}H_{19}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 0.93 and 1.02 (2 s, 6H, gem-dimethyl), 1.94 and 2.15 (2d, 2H, J = 15.5 Hz, H_6), 2.38 and 2.49 (2d, 2H, J = 17 Hz, H_8), 4.83 (s, 1H, H_{10}), 6.67 and 6.88 (2 s, 2H, H_4 and H_5-imidazole), 7.27–7.42 (m, 4H, $H_{1\text{-}4}$), 11.01 (brs, 1H, NH–DHP), 13.24 (s, 1H, NH-imidazole); MS: m/z (%) 345 (M^+, 64), 315 (9), 277 (28), 261 (56), 219 (35), 164 (39), 101 (20), 82 (27), 69 (50), 55 (100); IR (KBr) ν (cm^{-1}): 3293, 3088, 2945, 1680, 1644, 1511, 1368. Anal. Calcd. for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17; Found: C, 73.31; H, 5.21; N, 12.42.

*7,7-Dimethyl-10-(1-methyl-1H-imidazol-5-yl)-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**26**) ($C_{22}H_{21}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.03 and 1.08 (2 s, 6H, gem-dimethyl), 2.11 and 2.26 (2d, 2H, J = 16 Hz, H_6), 2.54 and 2.63 (2d, 2H, J = 17 Hz, H_8), 3.84 (s, 3H, N–CH_3), 4.62 (s, 1H, H_{10}), 6.43 (s, 1H, H_4-imidazole), 7.25–7.46 (m, 4H, $H_{1\text{-}4}$), 7.55 (s, 1H, H_2-imidazole), 10.49 (brs, 1H, NH); MS: m/z (%) 359 (M^+, 100), 329 (72), 275 (28), 246 (43), 233 (20), 164 (7), 101 (20), 55 (9); IR (KBr) ν (cm^{-1}): 3513, 3263, 3160, 3052, 2960, 1680, 1639, 1506, 1358. Anal. Calcd. for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.64; Found: C, 73.27; H, 5.65; N, 11.36.

*10-(4,5-Dichloro-1H-imidazol-2-yl)-7,7-dimethyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**27**) ($C_{21}H_{17}Cl_2N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.04 and 1.07 (2 s, 6H, gem-dimethyl), 2.13 and 2.25 (2d, 2H, J = 16 Hz, H_6), 2.57 and 2.61 (2d, 2H, J = 17 Hz, H_8), 4.70 (s, 1H, H_{10}), 7.28–7.59 (m, 4H, $H_{1\text{-}4}$), 10.53 (brs, 1H, NH-DHP), 13.09 (s, 1H, NH-imidazole); MS: m/z (%) 417 $[M+4)^+$, 1], 415{$(M+2)^+$, 6], 414 (M^+, 9), 329 (17), 278 (16), 227 (15), 167 (31), 137 (38), 108 (39), 83 (58), 60 (100); IR (KBr) ν (cm^{-1}): 3144, 3088, 3032, 2848, 1751, 1705, 1624, 1363, 758. Anal. Calcd. for $C_{21}H_{17}Cl_2N_3O_2$: C, 66.88; H, 4.14; N, 10.14; Found: C, 66.98; H, 4.37; N, 10.41.

*7,7-Dimethyl-10-(1-methyl-2-(methylthio)-1H-imidazol-5-yl)-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**28**) ($C_{23}H_{23}N_3O_2S$)*

^{1}H-NMR ($CDCl_3$): δ 1.01 and 1.03 (2 s, 6H, gem-dimethyl), 2.16 (2d, 2H, J = 16 Hz, H_6), 2.49 (2d, 2H, J = 17 Hz, H_8), 3.29 (s, 3H, S–CH_3), 3.76 (s, 3H, N–CH_3), 4.59 (s, 1H, H_{10}), 6.48 (s, 1H, C4-imidazole), 7.24–7.44 (m, 4H, $H_{1\text{-}4}$), 10.46 (brs, 1H, NH); MS: m/z (%) 405 (M^+, 100), 390 (55), 358 (29), 330 (14), 278 (10), 221 (11), 194 (13), 128 (14); IR (KBr) ν (cm^{-1}): 3447, 3147, 3036, 2949, 2852, 1681, 1641, 1508, 1361. Anal. Calcd. for $C_{23}H_{23}N_3O_2S$: C, 68.12; H, 5.72; N, 10.36; Found: C, 67.86; H, 5.48; N, 10.08.

*7,7-Dimethyl-10-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-phenyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**29**) ($C_{28}H_{24}N_4O_4$)*

^{1}H-NMR ($CDCl_3$): δ 1.00 and 1.02 (2 s, 6H, gem-dimethyl), 2.18 (2d, 2H, J = 16 Hz, H_6), 2.24 (2d, 2H, J = 17 Hz, H_8), 4.42 (s, 3H, N-CH_3), 5.11 (s, 1H, H_{10}), 6.86–7.42 (m, 4H, $H_{1\text{-}4}$), 7.61–7.67 (m, 5H, N-phenyl), 7.89 (s, 1H, H_4-imidazole); MS: m/z (%) 480 (M^+, 35), 464 (14), 434 (7), 354 (100), 298 (7), 270 (13); IR (KBr) ν (cm^{-1}): 3119, 2960, 2873, 1685, 1650, 1531, 1368. Anal. Calcd. for $C_{28}H_{24}N_4O_4$: C, 69.99; H, 5.03; N, 11.66; Found: C, 69.74; H, 5.29; N, 11.87.

*10-(1H-imidazol-2-yl)-7,7-dimethyl-5-phenyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**30**) ($C_{27}H_{23}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 0.99 and 1.02 (2 s, 6H, gem-dimethyl), 2.13 (2d, 2H, J = 16 Hz, H_6), 2.34 (2d, 2H, J = 17 Hz, H_8), 5.30 (s, 1H, H_{10}), 6.83–7.12 (m, 4H, $H_{1\text{-}4}$), 7.36–7.38 (2 s, 2H, H_4 and H_5-imidazole), 7.56–7.66 (m, 5H, N-phenyl), 9.70 (s, 1H, NH-imidazole); MS: m/z (%)

421 (M^+, 100), 393 (13), 354 (21), 345 (84), 316 (14), 270 (22), 193 (35), 169 (20), 77 (27), 45 (42); IR (KBr) ν (cm^{-1}): 3252, 3048, 2955, 1690, 1639, 1588, 1368. Anal. Calcd. for $C_{27}H_{23}N_3O_2$: C, 76.94; H, 5.50; N, 9.97; Found: C, 77.18; H, 5.76; N, 9.68.

*10-(4,5-Dichloro-1H-imidazol-2-yl)-7,7-dimethyl-5-phenyl-7,8-dihydro-5H-indeno[1,2-b]quinoline-9,11(6H,10H)-dione (**31**) ($C_{27}H_{21}Cl_2N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.02 (s, 6H, gem-dimethyl), 2.12 (2d, 2H, J = 20 Hz, H_6), 2.33 (2d, 2H, J = 18 Hz, H_8), 5.20 (s, 1H, H_{10}), 6.84–7.39 (m, 4H, H_{1-4}), 7.54–7.66 (m, 5H, N-phenyl), 9.98 (s, 1H, NH-imidazole); MS: m/z (%) 493 [$(M+4)^+$, 3], 491 [$(M+2)^+$, 18], 489 (M^+, 27), 472 (36), 412 (12), 355 (14), 338 (29), 293 (13), 241 (21), 136 (20), 104 (29), 78 (79), 46 (100); IR (KBr) ν (cm^{-1}): 3222, 3068, 2960, 1690, 1634, 1552, 1363, 768. Anal. Calcd. for $C_{27}H_{21}Cl_2N_3O_2$: C, 66.13; H, 4.32; N, 8.57; Found: C, 66.42; H, 4.58; N, 8.32.

General procedure for the synthesis of aromatized indeno[1,2-b]quinoline-9,11-diones, **32–40**

As it is illustrated in Fig. 2, all aromatized indeno[1,2-b]quinoline-9,11-dione*s* derivatives (**32–40**) were synthesized via oxidation of indeno[1,2-b]quinoline-9,11-dione substrates (compounds **19–31**). For this purpose, 1 equiv. of substrates was stirred with 5–10 equiv. of MnO_2 in dichloromethane at room temperature. The termination of the reaction was monitored by TLC (5–30 min) and the obtained product was filtered, washed with solvent and dried to obtain the desired aromatized compounds in moderate to good yields (16–70 %).

*10-(1-Methyl-5-nitro-1H-imidazol-2-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**32**) ($C_{20}H_{14}N_4O_4$)*

^{1}H-NMR ($CDCl_3$): δ 1.26 (m, 2H, H_7), 2.49 (t, 2H, H_6), 2.69 (t, 2H, H_8), 3.72 (s, 3H, N–CH_3), 7.63–7.89 (m, 4H, H_{1-4}), 8.12 (s, 1H, H_4-imidazole); MS: m/z (%) 374 (M^+, 29), 372 (86), 328 (27), 285 (100), 203 (28), 162 (36), 148 (14), 53 (21); IR (KBr) ν (cm^{-1}): 3421, 3135, 1716, 1690, 1552, 1378. Anal. Calcd. for $C_{20}H_{14}N_4O_4$: C, 64.17; H, 3.77; N, 14.97; Found: C, 64.02; H, 3.98; N, 14.68.

*10-(1H-imidazol-2-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**33**) ($C_{19}H_{13}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.24 (m, 2H, H_7), 2.12 (t, 2H, H_6), 2.63 (t, 2H, H_8), 7.63–7.78 (m, 4H, H_{1-4}), 7.93–7.95 (2 s, 2H, H_4 and H_5-imidazole), 12.20 (brs, 1H, NH-imidazole); MS: m/z (%) 315 (M^+, 50), 287 (100), 230 (29), 163 (28), 101 (36), 55 (34); IR (KBr) ν (cm^{-1}): 3411, 3140, 3058, 2963, 1709, 1687, 1360. Anal. Calcd. for $C_{19}H_{13}N_3O_2$: C, 77.37; H, 4.16; N, 13.33; Found: C, 77.64; H, 4.39; N, 13.07.

*10-(1-Methyl-1H-imidazol-5-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**34**) ($C_{20}H_{15}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.24 (m, 2H, H_7), 2.13 (t, 2H, H_6), 2.63 (t, 2H, H_8), 3.25 (s, 3H, N–CH_3), 6.78 (s, 1H, H_4-imidazole), 7.61–7.78 (m, 4H, H_{1-4}), 7.95 (s, 1H, H_2-imidazole); MS: m/z (%) 329 (M^+, 85), 312 (27), 272 (20), 245 (11), 190 (22), 123 (19), 111 (28), 96 (35), 81 (77), 69 (100); IR (KBr) ν (cm^{-1}): 2945, 1721, 1685, 1547. Anal. Anal. Calcd. for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.59; N, 12.76; Found: C, 73.16; H, 4.27; N, 12.46.

*10-(4,5-Dichloro-1H-imidazol-2-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**35**) ($C_{19}H_{11}Cl_2N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.24 (m, 2H, H_7), 2.14 (t, 2H, H_6), 2.65 (t, 2H, H_8), 7.48–7.92 (m, 4H, H_{1-4}), 13.40 (brs, 1H, NH-imidazole); MS: m/z (%) 387 [$(M+4)^+$, 5], 385 [$(M+2)^+$, 28] 383 (M^+, 42), 355 (100), 293 (14), 265 (15), 248 (64), 202 (13), 102 (53), 45 (14); IR (KBr) ν (cm^{-1}): 3221, 2955, 2919, 1716, 1685, 1557, 773. Anal. Calcd. for $C_{19}H_{11}Cl_2N_3O_2$: C, 59.39; H. 2.89; N, 10.94; Found: C, 59.12; H, 3.07; N, 11.12.

*7,7-Dimethyl-10-(1-methyl-5-nitro-1H-imidazol-2-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**36**) ($C_{22}H_{18}N_4O_4$)*

^{1}H-NMR ($CDCl_3$): δ 1.13 and 1.22 (2 s, 6H, gem-dimethyl), 1.55 (s, 2H, H_6), 2.56 (s, 2H, H_8), 3.71 (s, 3H, N–CH_3), 7.49–7.90 (m, 4H, H_{1-4}), 8.15 (s, 1H, H_4-imidazole); MS: m/z (%) 402 (M^+, 57), 316 (100), 245 (28), 217 (21), 191 (13), 54 (20); IR (KBr) ν (cm^{-1}): 2960, 2863, 1731, 1690, 1562, 1368. Anal. Calcd. for $C_{22}H_{18}N_4O_4$: C, 65.66; H, 4.51; N, 13.92; Found: C, 65.88; H, 4.73; N, 13.69.

*10-(1H-imidazol-2-yl)-7,7-dimethyl-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**37**) ($C_{21}H_{17}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.19 and 1.57 (2 s, 6H, gem-dimethyl), 2.79 (s, 2H, H_8), 3.10 (s, 2H, H_8), 7.89–7.91 (2 s, 2H, H_4 and H_5-imidazole), 7.49–7.75 (m, 4H, H_{1-4}), 12.25 (s, 1H, imidazole NH); MS: m/z (%) 343 (M^+, 93), 318 (82), 288 (100), 230 (14), 178 (6), 84 (8); IR (KBr) ν (cm^{-1}): 3411, 3150, 3053, 2950, 1716, 1685, 1557, 1368. Anal. Calcd. for $C_{21}H_{17}N_3O_2$: C, 73.45, H, 4.99, N, 12.24; Found: C, 73.19; H, 5.24; N, 12.51.

*7,7-Dimethyl-10-(1-methyl-1H-imidazol-5-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**38**) ($C_{22}H_{19}N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.14 and 1.20 (2 s, 6H, gem-dimethyl), 2.49 (s, 2H, H_6), 2.60 (s, 2H, H_8), 3.41 (s, 3H, N–CH_3), 6.91 (s, 1H, H_4-imidazole), 7.51–7.71 (m, 4H, H_{1-4}), 7.96 (s, 1H, H_2-imidazole); MS: m/z (%) 357 (M^+, 100), 342 (7), 302 (8), 273 (5); IR (KBr) ν (cm^{-1}): 3063, 2945, 2868, 1721, 1680, 1547. Anal. Calcd. for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76; Found: C, 74.17; H, 5.62; N, 11.48.

*10-(4,5-Dichloro-1H-imidazol-2-yl)-7,7-dimethyl-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**39**) ($C_{21}H_{15}Cl_2N_3O_2$)*

^{1}H-NMR ($CDCl_3$): δ 1.18 and 1.25 (2 s, 6H, gem-dimethyl), 2.77 (s, 2H H_8), 3.09 (s, 2H, H_8), 7.51–7.90 (m, 4H, H_{1-4}), 12.67 (s, 1H, NH–imidazole); MS: m/z (%) 415 [$(M+4)^+$, 11], 413 [$(M+2)^+$, 66], 4121 (M^+, 100), 354 (85), 292 (21), 264 (29), 202 (21), 176 (14), 83 (13), 56 (49); IR (KBr) ν (cm^{-1}): 3220, 2945, 2919, 1721, 1685, 1557, 764. Anal. Calcd. for $C_{21}H_{15}Cl_2N_3O_2$: C, 61.18; H, 3.67; N, 10.19; Found: C, 61.43; H, 3.89; N, 10.32.

*7,7-Dimethyl-10-(1-methyl-2-(methylthio)-1H-imidazol-5-yl)-7,8-dihydro-6H-indeno[1,2-b]quinoline-9,11-dione (**40**) ($C_{23}H_{21}N_3O_2S$)*

^{1}H-NMR ($CDCl_3$): δ 1.15 and 1.19 (2 s, 6H, gem-dimethyl), 2.57 (s, 2H, H_6), 2.68 (s, 2H, H_8), 3.21 (s, 3H, S–CH_3), 3.31 (s, 3H, N–CH_3), 6.99 (s, 1H, H_4-imidazole), 7.55–7.95 (m, 4H, H_{1-4}); MS: m/z (%) 403 (M^+, 100), 370 (85), 343 (21), 286 (14), 245 (12), 219 (7), 165 (7), 57 (6); IR (KBr) ν (cm^{-1}): 2950, 1716, 1670, 1552. Calcd. for $C_{23}H_{21}N_3O_2S$: C, 68.46; H, 5.25; N, 10.41; Found: C, 68.57; H, 5.01; N, 10.17.

Biological activity

Reagents and chemicals

RPMI 1640, fetal bovine serum (FBS), trypsin and phosphate buffered saline were purchased from Biosera (Ringmer, UK). 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) was obtained from Sigma (Saint Louis, MO, USA) and penicillin/streptomycin was purchased from Invitrogen (San Diego, CA, USA). Doxorubicin, *cis*-platin and dimethyl sulphoxide were obtained from EBEWE Pharma (Unterach, Austria) and Merck (Darmstadt, Germany), respectively.

Cell lines

HeLa (human cervical adenocarcinoma), LS180 (human colon adenocarcinoma), MCF-7 (human breast adenocarcinoma) and Jurkat (human T cell leukemia) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. All cell lines were maintained in RPMI 1640 supplemented with 10 % FBS, and 100 U/ml penicillin-G and 100 µg/ml streptomycin. Cells were grown in monolayer cultures, except for Jurkat cells, which were grown in suspension, at 37 °C in humidified air containing 5 % CO_2.

Antiproliferative effect

Cell viability following exposure to synthetic compounds was estimated by using the MTT reduction assay (Miri et al. 2004; Javidnia et al. 2005; Jabbar et al. 1989). MCF-7 and Jurkat cells were plated in 96-well microplates at a density of 5×10^4 cells/ml (100 µl per well). LS180 and HeLa cells were plated at densities of 1×10^5 and 2.5×10^4 cells/ml, respectively. Control wells contained no drugs and blank wells contained only growth medium for background correction. After overnight incubation at 37 °C, half of the growth medium was removed and 50 µl of medium supplemented with four different concentrations of synthetic compounds were added. All compounds were tested at the final concentration range of 1–100 µM except for compounds **19**, **20** and **29** which were tested at 1–25 µM and compounds **22**, **24** and **25**, which were tested in the range of 1–50 µM, due to lower solubility in DMSO. Plates with Jurkat cells were centrifuged before this procedure. Compounds were all first dissolved in DMSO and then diluted in medium so that the maximum concentration of DMSO in the wells was 0.5 %. Cells were further incubated for 72 h, except for HeLa cells, which were incubated for 96 h. At the end of the incubation time, the medium was removed and MTT was added to each well at a final concentration of 0.5 mg/ml and plates were incubated for another 4 h at 37 °C. Then formazan crystals were solubilized in 200 µl DMSO. The optical density was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680). The percentage of inhibition of viability compared to control wells was calculated for each concentration of the compound and IC_{16} and IC_{50} values (Tallarida and Murray 1987) were calculated with the software CurveExpert version 1.34 for Windows. The absorbance of wells containing no cells was subtracted from sample well absorbances before calculating the percentage of inhibition. Each experiment was repeated four times.

Results and discussion

Chemistry

The structures of all synthesized indeno[1,2-b]quinoline-9,11-diones were confirmed by spectroscopic and elemental analysis. The physical properties of synthesized compounds are summarized in Tables 1 and 2.

Antiproliferative activity

Prepared indeno[1,2-b]quinoline-9,11-dione derivatives were assayed for their antiproliferative activity in four human cancer cell lines (HeLa, LS180, MCF-7 and Jurkat) in terms of their IC_{16} and IC_{50} values (Table 3). In HeLa cell line, the most potent compound was **29** (IC_{16} = 9.2 μM). The order of decreasing activity in LS180 cell line was found to be **27** > **21** (IC_{16} values). This order was **22** > **39** > **31** for assayed compounds in MCF-7 cell line (IC_{16} values). In Jurkat cell lines the most potent compound in terms of its IC_{16} value was **29** followed by **31** and **33**.

Considering the data in Table 3, following rationales may be developed:

1. Indeno[1,2-b]quinoline-9,11-diones derivatives exhibited higher potency in Jurkat cancer cell lines compared to LS180, HeLa and MCF-7 cell lines.
2. Indeno[1,2-b]quinoline-9,11-dione derivatives were more potent in HeLa cell lines than LS180 cell lines.
3. Aromatization of the central ring in indeno[1,2-b]quinoline-9,11-diones resulted in reduced antiproliferative potential of compounds in LS180 and HeLa cell lines.
4. Although all synthesized indeno[1,2-b]quinoline-9,11-dione compounds possessed lower antiproliferative activity than Doxorubicin, but some compounds showed superior activity to *cis*-platin (compounds **29**, **31**, and **33** in Jurkat cell lines, **27** in LS180 cell line) (Table 3).
5. All of the compounds that exhibited superior antiproliferative activity to *cis*-platin in Jurkat cell lines, included imidazol-2-yl substituent on their DHP ring. This emphasizes the importance of this substitution on antiproliferative potential of indeno[1,2-b]quinoline-9,11-dione derivatives in Jurkat cell lines.

Table 3 Antiproliferative activity of indeno[1,2-b]quinoline-9,11-dione compounds assessed by the MTT reduction assay

Comp. No.	HeLa cells		LS180 cells		MCF-7 cells		Jurkat cells	
	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)
19	>25	>25	>25	>25	>25	>25	>25	>25
20	>25	>25	15.7 ± 2.5	>25	>25	>25	2.5 ± 1.6	>25
21	15.2 ± 2.9	>100	6.5 ± 0.5	66.7 ± 19.1	17.2 ± 5.4	>100	10.8 ± 1.6	>100
22	>50	>50	41.8 ± 12.1	>50	4.4 ± 0.9	>50	2.3 ± 0.7	>50
23	>100	>100	>100	>100	>100	>100	5.7 ± 1.0	>100
24	>50	>50	14.6 ± 2.9	>50	>50	>50	>50	>50
25	14.3 ± 2.5	>50	>50	>50	>50	>50	5.6 ± 2.1	>50
26	15.7 ± 2.2	>100	>100	>100	21.4 ± 4.8	>100	2.1 ± 0.2	41.7 ± 0.6
27	12.0 ± 2.1	>100	2.8 ± 0.5	23.5 ± 2.4	>100	>100	6.1 ± 2.5	61.0 ± 10.6
28	>100	>100	>100	>100	>100	>100	9.9 ± 5.4	>100
29	9.2 ± 1.1	>25	12.5 ± 3.5	>25	>25	>25	0.7 ± 0.1	>25
30	11.4 ± 2.9	>100	>100	>100	21.4 ± 4.5	>100	2.3 ± 1.5	>100
31	10.7 ± 2.7	59.0 ± 17.0	19.4 ± 1.3	>100	13.6 ± 2.4	77.1 ± 8.3	1.7 ± 0.8	95.8 ± 42.8
32	>100	>100	>100	>100	>100	>100	33.0 ± 1.5	>100
33	>100	>100	>100	>100	>100	>100	1.7 ± 1.1	70.9 ± 24.6
34	>100	>100	>100	>100	>100	>100	13.4 ± 11.1	>100
35	25.4 ± 5.7	>100	>100	>100	>100	>100	3.4 ± 0.8	51.5 ± 18.5
36	91.4 ± 82.5	>100	>100	>100	46.2 ± 26.6	>100	>100	>100
37	40.3 ± 5.3	>100	>100	>100	34.3 ± 27.7	>100	24.7 ± 16.0	>100
38	11.3 ± 2.0	>100	>100	>100	>100	>100	15.6 ± 8.6	>100
39	>100	>100	37.4 ± 8.1	>100	11.0 ± 1.3	>100	13.4 ± 8.0	>100
40	>100	>100	>100	>100	>100	>100	14.7 ± 1.1	>100
Doxorubicin	0.042 ± 0.007	0.281 ± 0.039	0.012 ± 0.003	0.118 ± 0.012	0.015 ± 0.003	0.326 ± 0.095	0.025 ± 0.004	0.192 ± 0.049
Cisplatin	5.6 ± 1.5	16.3 ± 2.3	5.0 ± 1.5	43.3 ± 9.2	4.0 ± 1.1	82.7 ± 24.8	1.9 ± 0.6	11.4 ± 3.2

6. It seems that introduction of the phenyl moiety on the nitrogen atom of the central ring may enhance antiproliferative effect of compounds in most cases (**22**: $IC_{16} = 4.4$ μM, MCF-7 cell line and $IC_{16} = 2.3$ μM, Jurkat cell line; **23**: $IC_{16} = 5.7$ μM, Jurkat cell line; **29**: $IC_{16} = 0.7$ μM, Jurkat cell line and $IC_{16} = 9.2$ μM, HeLa cell line; **30**: $IC_{16} = 2.3$ μM, Jurkat cell line; **31**: 1.7 μM, Jurkat cell line and $IC_{16} = 19.4$ μM, LS180 cell line). This finding is in agreement with previous reported results.
7. The introduction of electron-withdrawing groups such as nitro or chlorine on imidazole moiety positioned at central part of the indeno[1,2-b]quinoline-9,11-dione scaffolds, played an important role in antiproliferative potency of these compounds (**27**, **29** and **31**, this effect was less pronounced for MCF-7 cell lines). For example, in Jurkat cell line, compounds bearing nitro (**29**) or two chlorine (**31**) substituents showed superior activity over their analogues bearing no electronegative groups (Table 3). However; this finding confirmed our previous results (Miri et al. 2011).

Synthesized indeno[1,2-b]quinoline-9,11-diones were found to possess weak to relatively good antiproliferative activities in HeLa, LS180, MCF-7 and Jurkat human cancer cell lines. Some of the compounds exhibited superior antiproliferative activity to *cis*-platin especially in Jurkat cell lines. The results of the present study may be appropriate for further rational design of more potent anti-tumor agents based on the indeno[1,2-b]quinoline-9,11-dione scaffold. However considering the flattened structure of these compounds, one possible antiproliferative mechanism may be the DNA intercalation.

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