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MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Antiproliferative, DNA cleavage, and ADMET study of substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid and its esters

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Abstract: Synthesis, anti-proliferative, DNA cleavage, and *in silico* ADMET studies of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acids and their resultant esters in acid catalyzed medium have been investigated. The synthesized compounds are characterized by UV, IR, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. The electrophoretic DNA cleavage studies on λ-DNA (Eco-RI/Hinda-III double digest) using agarose gel method and the antiproliferative activity was carried out by MTT assay on five different human cancer cell lines (Chronic Myelogenous Leukemia (K562), Breast Cancer (MCF-7), Cervical Cancer (HeLa), Colorectal Adino carcinoma (Colo 205), and Hepato cellular carcinoma (HepG2)). Doxorubicin is taken as standard for comparison. The cleavage study indicated that molecules (3b–6a and 7b–8c) did cleave the DNA completely with no trace of fragments. The molecules (6b, 6c and 7a) have appeared to cleave DNA partially and assessed by comparing the bands appeared in control and test compounds at 100 µg concentration. The MTT antiproliferative activity of the synthesized derivatives at a concentration of 10 mM screened that out of the five cancer cell lines tested, the compounds 8b (25.97%, MCF-7), 7a (25.36%, Colo 205), and 7b (24.22%, HePG) showed considerable degree of activity at a very low concentration. The molecules were active against MCF-7, Colo 205, and HepG. The molecules exhibited acceptable range in *in silico* ADMET prediction, significant

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N.D. Satyanarayan obtained his Ph D from the Department of Pharmaceutical Chemistry from Gulbarga University, Gulbarga, India and postdoctoral research from University of Sunderland, UK. Presently, he is working as an assistant professor at Department of Pharmaceutical Chemistry, Kuvempu University, Karnataka, India. His research interest is mainly into drug discovery from natural and synthetic products.

PUBLIC INTEREST STATEMENT

The deaths due to patients suffering from cancer have become an important issue and resistance to drugs used in the treatment has become a common obstacle. Many drugs with different structures and mechanisms fail to alleviate the problem. The present study involves synthesis, anti-proliferative, and DNA cleavage of benzofuran coupled quinoline-4-carboxylic acids and their esters. The in-silico ADMET studies for drug likeliness of the molecules has been undertaken to avoid testing of toxic compounds. The molecules tested were found to be active against MCF-7, Colo 205, and HepG cell lines. The study further provides identification of possible lead moiety as an antiproliferative agent(s). The incorporation of various organic groups (R) attached showed distinctive differences in the biological property and is useful in bringing out a potent lead molecule which can be processed into an anticancer drug to help mankind to lead a healthy life.

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DNA cleavage, and antiproliferative properties. The study further provides identification of possible lead moiety as an antiproliferative agent.

Subjects: Chemical Spectroscopy; Medicinal & Pharmaceutical Chemistry; Organic Chemistry

Keywords: cinchophen; quinoline-4-carboxylic acid; ADMET; DNA cleavage; antiproliferative and MTT assay; anticancer drug discovery

1. Introduction

The mortality of patients suffering from various types of cancer has become an important issue worldwide. Resistance to chemotherapeutic agents used in the treatment of cancers has become a common obstacle (Arancia, Molinari, Calcabrini, Meschini, & Cianfrigli, 2001; Li et al., 2001). Several drugs including tamoxifen (TAM), 5-flurouracil (5FU), adriamycin (ADR), and vincristine (VCR) with different structures and mechanisms of antitumor treatment fail to alleviate such problem (Arancia et al., 2001; Li et al., 2001). Due to several side effects, drug resistance, and malfunction of antitumor drugs to exert their effects in certain cases of cancers (Cree, Knight, Di Nicolantonio, Sharma, & Gulliford, 2002; Faneyte, Kristel, & van de Vijver, 2001; Uchiyama-Kokubu & Watanabe, 2001), the search for new chemotherapeutic agents of synthetic or natural origins is one of the hot topics nowadays.

1-(1-benzofuran-2-yl) ethanone is a vital heterocyclic compound, which not only acts as a key structural subunit in naturally occurring analogs that exhibit outstanding biological activities but also represent valuable building block in the synthesis of natural products (Bird & Cheeseman, 1984; Simpson, 1985). Benzofuran derivatives have displayed wide range of biological actions such as antibacterial (Fukai, Oku, Hano, & Terada, 2004), analgesic (Basawaraj, Ashok, & Rajendra Prasad, 2009; Stanislv, Petr, Jitka, Petr, & Ivan, 2000), anti-inflammatory (Basawaraj et al., 2009; Fukai et al., 2004), anticancer (Basawaraj et al., 2009; Hayakawa et al., 2004), and cardiovascular activity (Oka et al., 2001). The quinoline core system is an important structural fragment of a large number of synthetic compounds displaying biological activities such as antibacterial, antimalarial, antihypertensive, anti-asthmatic, and as anti-inflammatory (Bilker et al., 1998; Chen, Fang, Sheu, Hsu, & Tzeng, 2001; Larsen et al., 1996). Cinchophen (2-phenyl quinoline-4-carboxylic acid) was earlier synthesized and introduced as a uricosuric agent for the treatment of gout (Sternlieb & Eisman, 1957). Cinchophen analogs containing benzofuran nucleus with phenolic esters and amides have been reported from our laboratory as antimicrobial and antioxidant agent (Shankerrao, Bodke, Harishkumar, Baburao, & Satyanarayan, 2011; Shankerrao, Bodke, & Mety, 2013). Quinoline carboxylic acid and their analogs confirm wide assortment of medicinal properties including antitumor (Feng, Ding-Qiao, Kai-Ling, & Wei, 2006), antiviral (Granik et al., 1978) and antibacterial activities (Nazrullaev, Bessonova, & Akhmedkhodzhaeva, 2001). The quinoline carboxylic acid and their analogs that could imitate ellipticene, exhibit considerable antitumor activity due to its DNA intercalating properties (Knölker & Reddy, 2002). It has been shown that quinolines reveal antitumor activity due to the enlargement of stable complex with DNA (Aravinda, Bhojya Naik, & Prakash Naik, 2010).

Deoxyribonucleic acid (DNA) is the major target molecule for the majority of anticancer and antiviral therapies according to cell biology (Jiao, Wang, Sun, & Jian, 2005; Sigman, Graham, Aurora, & Stern, 1979). Literature survey exposed that when one biodynamic heterocyclic system coupled with another, a molecule with pronounced biological activity is formed (Giri, Hanumanagoud, & Basavaraja, 2010). Encouraged by these observations, as a part of our research program, we have aimed at emerging a new biologically active heterocycle containing benzofuran and quinoline moieties.

In the present work, we synthesized 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid by using 2-acetyl benzofuran and different substituted isatin. The carboxylic acid functionality of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid was further used in the synthesis of different esters using

different aliphatic alcohols in acidic medium. The title compounds have not been explored for DNA cleavage and antiproliferative activity till date. Therefore the present study was initiated with the aim of investigating the DNA cleavage and antiproliferative activities of the cinchophen esters. The preliminary *in silico* ADMET studies for the potential drug likeliness of the compounds were investigated.

2. Materials and method

2.1. Materials

The chemicals used were of synthetic grade. Melting points were determined in open capillary and are uncorrected. The purity of compounds and the progress of the reaction were checked on pre-coated silica gel TLC plates. The UV spectral data were recorded on UV-1800 SHIMADZU Spectrophotometer. IR spectra were recorded with KBr pellet method on Nicolet-Impact-410 FT-IR spectrometer. ^{1}H NMR spectra were recorded on a JEOL FT NMR (400 MHz) spectrometer with TMS as an internal standard. The chemical shifts are represented in δ units and the coupling constant *J* was measured in Hz. The mass spectra were recorded on a JEOL SX 102/DA- 6000 (10 kV) FAB mass spectrometer.

2.2. Method (Scheme 1)

2.2.1. Synthesis of 1-(1-benzofuran-2-yl) ethanone (1)

To an alcoholic KOH (33%, 20 ml) solution, salicylaldehyde (5.8 g, 0.047 mol) and chloroacetone (4.3 g, 0.047 mol) were added and kept for stirring vigorously for about 2–3 h maintaining a temperature 0–5°C. The resultant mixture was poured onto crushed ice. The separated solid was filtered and recrystallized from petroleum ether (60–80). The yield was 85%, M. Pt. 75–78°C.

Step-I: ***General procedure for the synthesis of substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid 3(a–c)***

To a mixture of 1-(1-benzofuran-2-yl) ethanone (1.8 g, 0.0113 mol) and substituted 1H-indole-2,3-dione (1.5 g, 0.0113 mol) in ethanol(10 ml), aqueous solution of KOH (33%) 5 ml was added, and the reaction mixture was stirred at 65–70°C for about 8–10hrs. The reaction mixture was extracted with ethyl acetate (2–3 times) and aqueous layer was poured onto crushed ice, acidified with 10 M HCl and the resulting mass was kept overnight, filtered, and dried to get yellow amorphous powder yield 80%.

Step-II: ***General procedure for the synthesis of substituted methyl/ethyl/n-butyl 2-(1-benzofuran-2-yl) quinolone-4-carboxylates (4a–c, 5a–c, 6a–c, 7a–c and 8a–c)***

Analogs of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acids were dissolved in sufficient quantities of methanol, ethanol, n-butanol, propanol, and isopropanol with the addition of catalytic amount of Conc. H_2SO_4, and the mixture was refluxed for about 9–10 h. The reaction mixture was cooled to room temperature and poured onto the crushed ice, filtered, washed with water, dried, and recrystallized from petroleum ether (60–80) and ethyl acetate (3:1).

2.3. Spectral details

2.3.1. Methyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylate **(4a)**

Yield: 83%. M. Pt. 115–118°C; UV λmax nm: 209 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,145 (C–O), 3,100 (C–H), 1,750 (C=O), 1,250 (C–N), 3,100 (C–H); ^{1}H NMR (DMSO-$d_{6,}$ 400 MHz) (δ ppm): 8.59–8.61 (d, 1H, *J* = *8* Hz), 8.50 (s, 1H), 8.17–8.19 (d, 1H, *J* = *8* Hz), 7.87–7.89 (t, 2H, *J* = *8* Hz), 7.72–7.74 (m, 3H, *J* = *8* Hz), 7.42–7.43 (t, 1H, *J* = 4 Hz), 7.32–7.34 (t, 1H, *J* = *8* Hz), 4.0 (s, 3H); ^{13}C NMR (DMSO-$d_{6,}$ 100 MHz) (δ ppm): 165.83, 154.98, 153.79, 148.37, 147.89, 136.06, 130.77, 129.60, 128.45, 128.20, 126.18,

125.27, 123.66, 123.44, 122.21, 118.80, 111.67, 107.27, 52.99. Calcd. 303.31 gm/ml. EI-MS (*m/z*): 304.0 (M^+1).

2.3.2. Methyl 2-(1-benzofuran-2-yl)-8-floroquinoline-4-carboxylate **(4b)**
Yield: 80%. M. Pt. 122–124°C; UV λmax nm: 194 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,140 (C–O), 3,100 (C–H), 1,755 (C=O), 1,250 (C–N), 3,100 (C–H), 1,400 (C–F); ^1H NMR (DMSO-d_6, 400 MHz) (δ ppm): 8.46 (s, 1H), 8.34–8.35 (t, 1H, *J* = 4 Hz), 7.86 (s, 1H), 7.72–7.74 (t, 2H, *J* = *8* Hz), 7.64–7.66 (t, 2H, *J* = *8* Hz), 7.410–7.413 (t, 1H, *J* = *1.2* Hz), 7.29–7.30 (t, 1H, *J* = 4 Hz), 4.01 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) (δ ppm): 165.41, 158.42, 155.87, 155.01, 153.40, 147.91, 138.48, 135.82, 128.10, 126.33, 124.91, 123.65, 122.27, 121.20, 119.73, 114.82, 111.66, 107.87, 53.06. Calcd. 321.30 gm/ml. EI-MS (*m/z*): 322.0 (M + 1).

2.3.3. Methyl 2-(1-benzofuran-2-yl)-6-chloroquinoline-4-carboxylate **(4c)**
Yield: 85%. M. Pt. 125–128°C; UV λmax nm: 194 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,145 (C–O), 3,100(C–H), 1,740 (C=O), 1,250 (C–N), 3,100 (C–H), 740 (C–Cl); ^1H NMR (DMSO-d_6, 400 MHz) (δ ppm): 8.66 (d, 1H, *J* = 4 Hz), 8.50 (s, 1H), 8.14–8.16 (d, 1H, *J* = *8* Hz), 7.86–7.87 (t, 2H, *J* = 4 Hz), 7.74–7.76 (t, 2H, *J* = *8* Hz), 7.42–7.44 (t, 1H, *J* = *8* Hz), 7.31–7.33 (t, 1H, *J* = *8* Hz), 4.03 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) (δ ppm): 165.29, 155.03, 153.40, 148.28, 146.90, 134.77, 133.08, 131.59, 131.21, 128.13, 126.35, 124.24, 124.15, 123.70, 122.28, 119.94, 111.68, 107.74, 53.11. Calcd. 337.75 gm/ml. EI-MS (*m/z*): 338.0 (M + 1).

2.3.4. Ethyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylate **(5a)**
Yield: 84%. M. Pt. 68–70°C; UV λmax nm: 212 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,145 (C–O), 3,100 (C–H), 1,755 (C=O), 1,250 (C–N); ^1H NMR (DMSO-d_6, 400 MHz) (δ ppm): 8.57–8.59 (d, 1H, *J* = *8* Hz), 8.48 (s, 1H), 8.17–8.19 (d, 1H, *J* = *8* Hz), 7.91 (d, 1H, *J* = 4 Hz), 7.87–7.89 (t, 1H, *J* = *8* Hz), 7.73–7.74 (m, 3H, *J* = 4 Hz), 7.43–7.45 (t, 1H, *J* = *8* Hz), 7.32–7.33 (t, 1H, *J* = 4 Hz), 4.49 (d, 5H, *J* = 4 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (δ ppm): 165.42, 154.97, 153.79, 148.34, 147.85, 136.45, 130.73, 129.59, 128.4, 126.16, 125.21, 123.64, 123.41, 122.18, 118.58, 111.65, 107.31, 61.99, 14.02. Calcd. 317.33 gm/ml. EI-MS (*m/z*): 318.0 (M + 1).

2.3.5. Ethyl 2-(1-benzofuran-2-yl) -8-fluoroquinoline-4-carboxylate **(5b)**
Yield: 80%. M. Pt. 80–82°C; UV λmax nm: 201 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,140 (C–O), 3,100 (C–H), 1,755 (C=O), 1,250 (C–N), 3,100 (C–H), 1,400 (C–F); ^1H NMR (DMSO-d_6, 400 MHz) (δ ppm): 8.52 (s, 1H), 8.36–8.38 (t, 1H, *J* = *8* Hz), 7.95 (s, 1H), 7.76–7.78 (t, 2H, *J* = *8* Hz), 7.69–7.70 (t, 2H, *J* = 4 Hz), 7.43–7.45 (t, 1H, *J* = *8* Hz), 7.32–7.34 (t, 1H, *J* = *8* Hz), 4.48–4.50 (t, 2H, *J* = *8* Hz), 1.42–1.44 (t, 3H, *J* = 4 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (δ ppm): 165.12, 158.48, 155.93, 153.47, 148.00, 138.61, 136.48, 128.14, 126.41, 124.93, 123.71, 122.33, 121.27, 119.61, 114.75, 111.72, 108.06, 62.19, 13.99. Calcd. 335.32 gm/ml. EI-MS (*m/z*): 336.0 (M + 1).

2.3.6. Ethyl 2-(1-benzofuran-2-yl) -6-chloroquinoline-4-carboxylate **(5c)**
Yield: 85%. M. Pt. 84–85°C; UV λmax nm: 204 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,145 (C-O), 3,100 (C–H), 1,740 (C=O), 1,325 (C–N), 1,300 (C–H), 740 (C–Cl); ^1H NMR (DMSO-d_6, 400 MHz) (δ ppm): 8.67–8.68 (d, 1H, *J* = 4 Hz), 8.51 (s, 1H), 8.16–8.18 (d, 1H, *J* = *8* Hz), 7.88–7.90 (t, 3H, *J* = *8* Hz), 7.75–7.77 (t, 3H, *J* = *8* Hz), 7.433–7.436 (t, 1H, *J* = *1.2* Hz), 7.32–7.34 (t, 1H, *J* = *8* Hz), 4.48–4.47 (q, 3H, *J* = 4 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (δ ppm): 164.86, 155.05, 153.44, 148.31, 146.91, 135.16, 133.04, 131.63, 131.23, 128.14, 126.37, 123.72, 124.24, 122.29, 119.83, 111.70, 107.84, 62.86, 13.99. Calcd. 351.78 gm/ml. EI-MS (*m/z*): 352.0 (M + 1).

2.3.7. Butyl 2-(1-benzofuran-2-yl)-8-fluoroquinoline-4-carboxylate **(6a)**
Yield: 82%. M. Pt. 88–90°C; UV λmax nm: 205 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,145 (C–O), 3,100 (C–H), 1,750 (C=O), 1,230 (C–N), 3,100 (C–H), 1,400 (C–F); ^1H NMR (DMSO-d_6, 400 MHz) (δ ppm): 8.52 (s, 1H), 8.351–8.356 (t, 1H, *J* = 2 Hz), 7.955–7.957 (d, 1H, *J* = *0.8* Hz), 7.785–7.787 (t, 1H, *J* = *0.8* Hz), 7.70–7.704 (m, 2H, *J* = *1.6* Hz), 7.43–7.44 (t, 1H, *J* = 4 Hz), 7.32–7.33 (t, 1H, *J* = 4 Hz), 4.45–4.46 (t, 2H, *J* = *8* Hz), 1.77–1.78 (m, 2H, *J* = 4 Hz), 1.42–1.43 (m, 2H, *J* = 4 Hz), 1.24–1.26 (d, 1H, *J* = *8* Hz), 0.95–0.97

(t, 3H, *J* = *8* Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (*δ* ppm): 165.50, 155.06, 153.45, 148.02, 136.55, 128.41, 128.13, 126.42, 124.93, 123.72, 122.35, 121.20, 119.59, 114.95, 114.77, 111.72, 108.07, 65.83, 30.03, 18.68, 13.56. Calcd. 363.38 gm/ml. EI-MS (*m/z*): 364.0 (M + 1).

2.3.8. Butyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylate **(6b)**
Yield: 87%. M. Pt. 76–78°C; UV *λ*max nm: 201 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,140 (C–O), 3,100(C–H), 1,755 (C=O), 1,180 (C–N); ^{1}H NMR (DMSO-d_6, 400 MHz) (*δ* ppm): 8.550–8.552 (d, 1H, *J* = *0.8* Hz), 8.45 (s, 1H), 8.16–8.18 (d, 1H, *J* = *8* Hz), 7.86–7.87 (m, 2H, *J* = 4 Hz), 7.720–7.723 (m, 3H, *J* = *1.2* Hz), 7.426–7.429 (t, 1H, *J* = *1.2* Hz), 7.322–7.324 (t, 1H, *J* = *0.8* Hz), 4.45–4.47 (t, 2H, *J* = *8* Hz), 1.77–1.79 (m, 2H, *J* = *8* Hz), 1.43–1.45 (m, 2H, *J* = *8* Hz), 0.95–0.97 (t, 3H, *J* = *8* Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (*δ* ppm): 165.50, 154.97, 153.78, 148.35, 147.86, 136.49, 129.61, 130.75, 128.19, 126.17, 125.18, 123.64, 123.40, 122.20, 118.56, 111.65, 107.31, 65.64, 30.06, 18.69, 13.55. Calcd. 345.39 gm/ml. EI-MS (*m/z*): 346.0 (M + 1).

2.3.9. Butyl 2-(1-benzofuran-2-yl)-6-chloroquinoline-4-carboxylate **(6c)**
Yield: 84%. M. Pt. 92–94°C; UV *λ*max nm: 192 (methanol); IR (KBr), cm^{-1} 1,610 (C=C), 1,145 (C–O), 3,100 (C–H), 1,740 (C=O), 1,230 (C–N), 1,300 (C–H), 740 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) (*δ* ppm): 8.67–8.68 (d, 1H, *J* = 4 Hz), 8.52 (s, 1H), 8.18–8.20 (d, 1H, *J* = *8* Hz), 7.89–7.90 (m, 3H, *J* = 4 Hz), 7.75–7.76 (m, 3H, *J* = 4 Hz), 7.43–7.44 (m, 2H, *J* = 4 Hz), 7.32–7.33 (t, 2H, *J* = 4 Hz), 4.46–4.47 (t, 3H, *J* = 4 Hz), 1.78–1.79 (m, 3H, *J* = 4 Hz), 1.46–1.48 (m, 3H, *J* = *8* Hz), 0.96–0.98 (t, 5H, *J* = *8* Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (*δ* ppm): 165.26, 155.03, 148.33, 146.94, 135.27, 133.03, 131.65, 131.24, 126.38, 123.73, 124.28, 122.30, 119.85, 111.70, 107.85, 65.82, 30.61, 18.71, 13.55. Calcd. 379.83 gm/ml. EI-MS (*m/z*): 380.0 (M + 1).

2.3.10. Propyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylate **(7a)**
Yield: 79%. M. Pt. 78–80°C; IR (KBr), cm^{-1} 1,547.40 (C=C), 1,146.02 (C–O), 2,975.37 (C–H), 1,716.96 (C=O), 1,246.93 (C–H); ^{1}H NMR (DMSO-d_6, 400 MHz) (*δ* ppm): 8.526–8.528 (d, 1H, *J* = *0.8* Hz), 8.420 (s, 1H), 8.163–8.165 (d, 1H, *J* = *0.8* Hz), 7.924–7.926 (d, 1H, *J* = *0.8* Hz), 7.864–7.867 (m, 1H, *J* = *1.2* Hz), 7.756–7.759 (d, 2H, *J* = *1.2* Hz), 7.71–7.72 (d, 1H, *J* = 4 Hz), 7.425–7.428 (d, 1H, *J* = *1.2* Hz), 7.321–7.323 (d, 1H, *J* = *1.2* Hz), 5.31–5.33 (m, 1H, *J* = *8* Hz), 1.44–1.46 (d, 6H, *J* = *8* Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (*δ* ppm): 165.09, 155.03, 148.35, 147.89, 130.82, 129.67, 126.25, 125.21, 123.72, 122.26, 118.45, 111.75, 107.49, 70.01, 21.59. Calcd. 331.36 gm/ml. EI-MS (*m/z*): 332.10 (M + 1).

2.3.11. Propyl 2-(1-benzofuran-2-yl)-8-fluoroquinoline-4-carboxylate **(7b)**
Yield: 83%. M. Pt. 122–123°C; IR (KBr), cm^{-1} 1,595.93 (C=C), 1,147.53 (C–O), 2,962.17 (C–H), 1,716.59 (C=O), 1,248.09 (C–H), 1,202.47 (C–F); ^{1}H NMR (DMSO-d_6, 400 MHz) (*δ* ppm): 8.48 (s, 1H), 8.31–8.32 (t, 1H, *J* = 4 Hz), 7.965–7.967 (d, 1H, *J* = *0.8* Hz), 7.766–7. 768 (m, 1H, *J* = *0.8* Hz), 7.67–7.69 (d, 2H, *J* = *8* Hz), 7.43–7.44 (t, 2H, *J* = 4 Hz), 7.327–7.329 (d, 1H, *J* = *0.8* Hz), 5.29–5.31 (m, 1H, *J* = *8* Hz), 1.44–1.46 (d, 6H, *J* = *8* Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (*δ* ppm): 164.77, 158.28, 156.24, 155.11, 153.50, 148.03, 136.99, 128.18, 126.48, 123.78, 122.40, 119.48, 111.80, 108.23, 70.26, 21.56. Calcd. 349.35 gm/ml. EI-MS (*m/z*): 350.10 (M + 1).

2.3.12. Propyl 2-(1-benzofuran-2-yl)-6-chloroquinoline-4-carboxylate **(7c)**
Yield: 82%. M. Pt. 133–134°C; IR (KBr), cm^{-1} 1,546.96 (C=C), 1,145.42 (C–O), 2,957.50 (C–H), 1,714.61 (C=O), 1,242. 90 (C–H), 751.08 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) (*δ* ppm): 8.661–8.667 (d, 1H, *J* = *2.4* Hz), 8.48 (s, 1H), 8.17–8.19 (d, 1H, *J* = *8* Hz), 7.956–7.958 (d, 1H, *J* = *0.8* Hz), 7.891–7.897 (d, 1H, *J* = *2.4* Hz), 7.760–7.762 (t, 2H, *J* = *0.8* Hz), 7.436–7.439 (d, 1H, *J* = *1.2* Hz), 7.32–7.33 (d, 1H, *J* = 4 Hz), 5.30–5.31 (m, 1H, *J* = 4 Hz), 1.32–1.35 (d, 7H, *J* = *8* Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) (*δ* ppm): 164.46, 155.10, 153.48, 146.96, 126.45, 124.28, 123.80, 122.36, 119.77, 111.78, 108.02, 70.22, 21.57. Calcd. 365.80 gm/ml. EI-MS (*m/z*): 366.10 (M + 1).

2.3.13. Propan-2-yl 2-(1-benzofuran-2-yl) quinoline-4-carboxylate **(8a)**
Yield: 76%. M. Pt. 79–80°C; IR (KBr), cm^{-1} 1,548.01 (C=C), 1,151.43 (C–O), 2,975.45 (C–H), 1,721.11 (C=O), 1,250.13 (C–H); ^{1}H NMR (DMSO-d_6, 400 MHz) (*δ* ppm): 8.526–8.528 (d, 1H, *J* = *0.8* Hz),

8.164–8.165 (d, 1H, *J* = *0.4* Hz), 7.924–7.927 (d, 1H, *J* = *1.2* Hz), 7.864–7.868 (t, 1H, *J* = *1.6* Hz), 7.757–7.759 (d, 2H, *J* = *0.8* Hz), 7.425–7.428 (d, 1H, *J* = *1.2* Hz), 7.321–7.324 (d, 1H, *J* = *1.2* Hz), 5.33–5.35 (m, 1H, *J* = *8* Hz), 1.44–1.46 (d, 6H, *J* = *8* Hz); ^{13}C NMR (DMSO-$d_{6,}$ 100 MHz) (δ ppm): 165.09, 155.02, 148.34, 147.89, 130.82, 129.67, 126.25, 125.21, 123.72, 122.26, 118.45, 111.75, 107.48, 70.01, 21.59. Calcd. 331.36 gm/ml. EI-MS (*m/z*): 332.20 (M + 1).

2.3.14. Propan-2-yl 2-(1-benzofuran-2-yl)-8-fluoroquinoline-4-carboxylate **(8b)**
Yield: 81%. M. Pt. 118–120°C; IR (KBr), cm^{-1} 1,553.96 (C=C), 1,147.51 (C–O), 2,963.58 (C–H), 1,716.49 (C=O), 1,247.73 (C–H), 1,202.30 (C–F); ^{1}H NMR (DMSO-$d_{6,}$ 400 MHz) (δ ppm): 8.48 (s, 1H), 8.31–8.32 (t, 1H, *J* = 4 Hz), 7.966–7.968 (d, 1H, *J* = *0.8* Hz), 7.767–7.769 (d, 2H, *J* = *0.8* Hz), 7.700–7.703 (d, 2H, *J* = *1.2* Hz) 7.43–7.44 (t, 1H, *J* = 4 Hz), 7.32 (d, 1H, *J* = 4 Hz), 5.29–5.31 (m, 1H, *J* = *8* Hz), 1.44–1.46 (d, 6H, *J* = *8* Hz); ^{13}C NMR (DMSO-$d_{6,}$ 100 MHz) (δ ppm): 164.77, 158.28, 156.25, 155.11, 153.50, 148.03, 136.98, 128.19, 126.49, 123.79, 122.40, 119.48, 111.80, 108.23, 70.26, 21.56. Calcd. 349.35 gm/ml. EI-MS (*m/z*): 350.10 (M + 1).

2.3.15. Propan-2-yl 2-(1-benzofuran-2-yl)-6-chloroquinoline-4-carboxylate **(8c)**
Yield: 74%. M. Pt. 129–130°C; IR (KBr), cm^{-1} 1,546.82 (C=C), 1,145.23 (C–O), 2,980.68 (C–H), 1,715.30 (C=O), 1,242.15 (C–H), 750.10 (C–Cl); ^{1}H NMR (DMSO-$d_{6,}$ 400 MHz) (δ ppm): 8.661–8.667 (d, 1H, *J* = *2.4* Hz), 8.48 (s, 1H), 8.174 (d, 1H, *J* = *0.4* Hz), 7.956 (d, 1H, *J* = *0.8* Hz), 7.891–7.897 (d, 1H, *J* = *2.4* Hz), 7.805–7.807 (t, 1H, *J* = *0.8* Hz), 7.781–7.783 (t, 1H, *J* = *0.8* Hz), 7.760–7.762 (t, 1H, *J* = *0.8* Hz), 7.436–7.439 (d, 1H, *J* = *1.2* Hz), 7.328–7.331 (d, 1H, *J* = *1.2* Hz), 5.33–5.34 (m, 1H, *J* = 4 Hz), 1.45–1.46 (d, 7H, *J* = 4 Hz); ^{13}C NMR (DMSO-$d_{6,}$ 100 MHz) (δ ppm): 164.46, 155.10, 153.48, 148.34, 131.71, 131.29, 126.45, 124.28, 123.80, 122.36, 119.77, 111.79, 108.03, 70.22, 21.57. Calcd. 365.80 gm/ml. EI-MS (*m/z*): 366.10 (M + 1).

3. Biological activity

3.1. ADME-toxicity prediction
The molecular descriptors of synthesized compounds (**4a–c, 5a–c, 6a–c, 7a–c, and 8a–c**) are predicted by pharmacokinetic parameters like absorption, distribution, metabolism, excretion, and toxicity (ADMET). The ADMET SAR (Feixiong et al., 2012**)** helps to evaluate biologically active molecules and eliminate biologically poor molecule, active lead molecule which contains undesirable functional groups based on Lipinski rule. The statistical calculation for lead molecules includes surface area, geometry, and fingerprint properties which help to understand biologically important end points. Aqueous solubility (PlogS), Blood brain barrier penetration (QlogBB), intestinal absorption (logHIA) (Lin & Yamazaki, 2003) and hepatotoxicity, Caco-2 cell permeability (QPPCaco) also help to predict the toxicity of lead molecules with intraperitoneal, oral, intravenous, and subcutaneous toxic effects of blood, cardiovascular system, gastrointestinal, kidney, liver, and lungs. The *in silico* study enables to decide the safety and efficacy of active molecules take up the molecule for in-depth studies.

3.2. DNA cleavage studies
The degree of DNA cleavage by the compounds was monitored by agarose gel electrophoresis method (Sambrook, Fritsch, & Maniatis, 1989). Agarose (0.25 gm) was weighed and dissolved in 25 ml of Tris acetate (TAE) buffer (50 mM, pH 8.0) and gel cassette was placed in the electrophoresis chamber inundated with TAE buffer. To this 20 µl of DNA sample along with bromophenol blue dye in 1:1 ratio with standard DNA marker was loaded. CT-DNA was treated with analogs (40 µM, 2 µl) followed by the dilution of buffer to a total volume of 20 µl. The samples after incubation at 37°C were loaded to the wells.

Electrophoretic mobility was achieved by supplying 50 V of electricity for about 45 min in TAE buffer. The gel along with platform was stained with 100 ml ethidium bromide (10 µg/ml) in sterile distilled water. Ethidium bromide binds to double stranded DNA by intercalation, because of steric hindrance for intercalation in covalently closed circular DNAs; it binds less than linear and open

Table 1. Antiproliferative activity of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid and its esters against different cancer cell lines

Code	K562	MCF-7	Hela	Colo	HepG2	HEK
3b	0.0	0.52	4.89	2.95	15.57	−2.90
3c	7.27	8.77	0.0	0.0	24.22	−5.69
4a	3.55	16.30	0.0	3.33	7.81	−7.72
4b	0.0	21.60	18.35	2.89	15.13	−12.75
4c	0.0	7.75	16.57	2.85	13.85	−10.73
5a	6.16	7.55	8.34	3.16	15.67	5.91
5b	1.22	7.24	15.79	1.94	21.08	−1.28
5c	0.0	9.58	16.35	3.37	19.95	−4.96
6a	0.0	8.26	11.79	16.69	18.67	−11.86
6b	0.0	12.03	9.34	1.73	14.74	−0.61
6c	5.64	11.72	8.12	0.0	17.78	−6.47
7a	0.0	14.06	8.67	0.0	15.67	6.25
7b	0.0	12.33	7.11	25.36	0.0	−0.05
7c	0.0	18.85	0.0	15.87	21.52	4.07
8a	0.0	22.82	0.77	11.86	7.17	3.40
8b	0.0	25.97	0.0	7.84	9.38	4.91
8c	5.55	12.84	0.0	18.61	6.28	−13.21

circles. After about 10–15 min the platform and the gel were rinsed with distilled water and gel was gently placed onto the UV transilluminator. The DNA bands appeared on the gel determined the cleavage by the entitled molecules tested.

3.3. MTT assay method for antiproliferative activity

The five human cancer cell lines and one normal cell line that were used in study are chronic myelogenous leukemia (K562), breast adenocarcinoma (MCF-7), cervical cancer (HeLa), colorectal adenocarcinoma (Colo 205), Hepato cellular carcinoma (HepG2), and normal human kidney embryonic cell line (HEK293) as represented in Table 1. The cells lines were obtained from the Department of National Centre of Cell Sciences, Pune, India, and were cultured at a seeding density of 0.2 × 106 in DMEM/RPMI medium supplemented with 100U/mL penicillin, 10% FBS, and 100 μg/mL streptomycin, respectively, and maintained in a humidified atmosphere with 5% CO2 at 37°C. The samples were dissolved in dimethyl sulfoxide (DMSO; not exceeding the final concentration of 0.01%) and further diluted in cell culture medium. The antiproliferative response of different molecules was assessed by MTT assay (Mosmann, 1983). Cells (~10,000) were plated in 200 μL growth medium in the presence or absence of the molecule (25, 50, 100, and 200 μg/mL) in 96-well culture plates for 24 h. Then the culture plates were centrifuged at 2,000 rpm for 10 min at room temperature. About 100 μL of supernatant was discarded and 20 μL of MTT (5 mg/mL in PBS) was added to each well and incubated for 4 h at 37°C. The viability of the cells was determined using a spectrophotometer at 570 nm. The IC50, that is, the concentration of the compound required to inhibit cell growth by 50%, was determined.

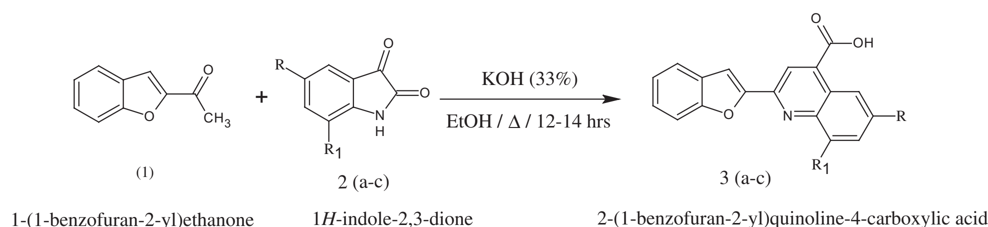
4. Results and discussion

4.1. Chemistry

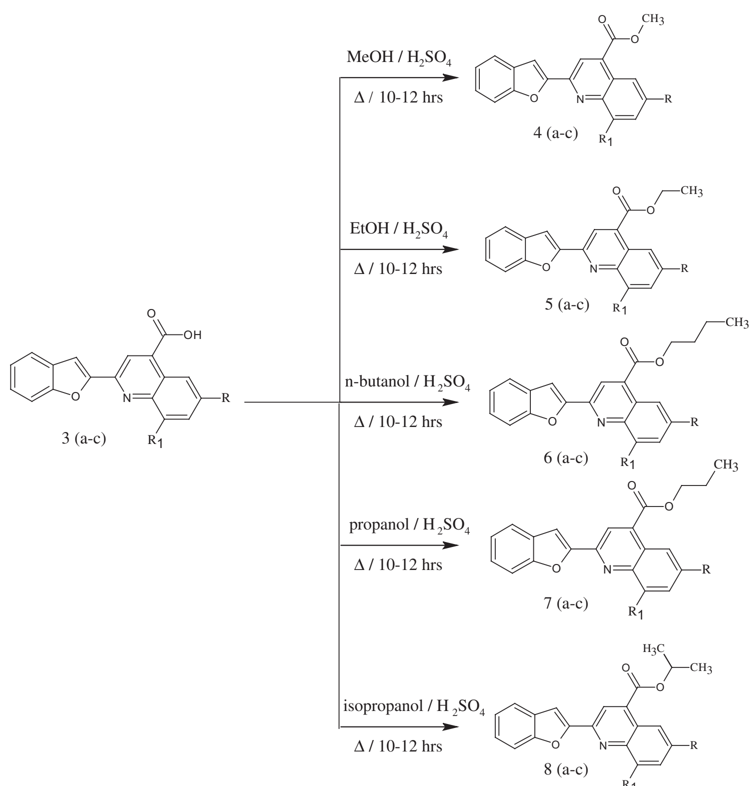
The synthesis of key intermediate 1-(1-benzofuran-2-yl) ethanone **(1)** and its utilization in the construction of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acids **3(a–c)** are shown in Scheme 1. The intermediate **(1)** was synthesized by stirring a mixture of 2-hydroxybenzaldehyde with chloroacetone in basic medium using methanol as solvent. Subsequently, 2-(1-benzofuran-2-yl)

Scheme 1. Synthesis of substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid, and its esters.

Step-I



Step-II



R	R_1
-H-	-F
-Cl	-H-
-H-	-H-

quinoline-4-carboxylic acids **3(a–c)** were synthesized by the reaction of compound **(1)** with substituted isatins 2(a–c) in basic medium to give the products **3(a–c).** The esters, methyl/ethyl/n-butyl/propyl/isopropyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylates **4(a–c)**, **5(a–c), 6(a–c) 7(a–c),** and **8(a–c)** were synthesized by reacting **3(a–c)** with different alcohols such as methanol, ethanol, n-butanol, propanol, and isopropanol in the presence of catalytic amount of Conc. H_2SO_4 to give the product in good yield.

IR spectra of the compounds showed absorption band with in 1,720–1,750 cm^{-1} due to the characteristic C=O group of all the different esters.

The ^{1}H NMR spectra of **4(a–c)** showed a singlet at δ 4.03–4.0 ppm corresponding to methyl protons of methyl ester, the remaining peaks which appeared at δ 8.61–7.32 ppm corresponding to Ar–H and

protons of the quinoline ring. The ^{13}C-NMR spectra of **4(a–c)** showed a peak at δ 165.83–165.29 ppm corresponding to C=O carbon of the ester, the peaks between δ 154.92 and 107.27 ppm corresponding to aromatic carbon, and the peak at δ 53.11–52.99 ppm corresponding to methyl ester carbon. MS analysis of **4(a-c)** displayed the molecular ion peak conforming their molecular weight.

The ^{1}H NMR spectra of **5(a–c)** show a quartet and triplet peak at δ 4.54–4.48 and 1.46–1.42 ppm corresponding to methylene and methyl protons of ethyl ester, the remaining peaks which appeared at δ 8.57–7.33 ppm corresponding to Ar–H and protons of the quinoline ring. The ^{13}C-NMR spectra of **5(a–c)** showed the peak at δ 165.42–164.86 ppm corresponding to C=O carbon of ester, the peak between δ 154.97 and 107.31 ppm corresponding to aromatic carbons, and the peak at δ 62.19–61.99 and 14.02–13.99 ppm corresponding to methylene and methyl carbons of ethyl ester. The MS analysis of **5(a–c)** displayed the molecular ion peak conforming their molecular weight.

The ^{1}H NMR spectra of **6(a–c)** show a triplet, quintet, sextet, and triplet peak at δ 4.49–4.46, 1.83–1.78, 1.50–1.42, and 1.0–0.97 ppm corresponding to methylene and methyl protons of n-butyl ester, the remaining peaks which appeared at δ 8.52–7.33 ppm corresponding to Ar–H and protons of the quinoline ring. The ^{13}C-NMR spectra of **6(a–c)** exhibited in each case a peak at δ 165.50–165.20 ppm corresponding to C=O carbon of ester, the peaks between δ 155.06 and 108.07 ppm corresponding to aromatic carbon, and the peak at δ 65.83–65.64, 30.06–30.01, 18.71–18.68, and 13.56–13.55 ppm corresponding to methylene and methyl carbons of n-butyl ester. MS analysis of **6(a–c)** displayed the molecular ion peak conforming their molecular weight.

^{1}H NMR spectra of **7(a–c)** show a multiplet and doublet peak at δ 5.39–5.30 and 1.46–1.44 ppm corresponding to methylene and methyl protons of propyl ester, the remaining peaks which appeared at δ 8.55–7.35 ppm corresponding to Ar–H and protons of the quinoline ring. The ^{13}C-NMR spectra of **7(a–c)** exhibited in each case a peak at δ 165.09–164.77 ppm corresponding to C=O carbon of ester, the peaks between δ 155.03–107.49 ppm corresponding to aromatic carbon, and the peak at δ 70.22–70.01, and 21.59–21.57 ppm corresponding to methylene and methyl carbons of propyl ester. MS analysis of **7(a–c)** displayed the molecular ion peak conforming their molecular weight.

^{1}H NMR spectra of **8(a–c)** show a septet and doublet peak at δ 5.36–5.31 and 1.46–1.44 ppm corresponding to methylene and methyl protons of isopropyl ester, the remaining peaks which appeared at δ 8.52–7.33 ppm corresponding to Ar–H and protons of the quinoline ring. The ^{13}C-NMR spectra of **8(a–c)** exhibited in each case a peak at δ 165.09–164.46 ppm corresponding to C=O carbon of ester, the peaks between δ 155.02–107.48 ppm corresponding to aromatic carbon and the peak at δ 70.26–70.01, and 21.59–21.56 ppm corresponding to methylene and methyl carbons of isopropyl ester. MS analysis of **8(a–c)** displayed the molecular ion peak conforming their molecular weight.

4.2. Pharmacokinetics properties

4.2.1. In silico ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles

The compounds with poor bioavailability show less effectiveness against disease. To solve this problem, predicting bioavailability properties will be of great advantage for drug development. Hence, using computer-based methods like ADMET and SAR tools the molecular descriptors and drug likeliness properties were studied. The pharmacokinetic properties are represented in Table 2. The coefficient of blood/brain barrier penetration (logB/B) was computed and assessed with central nervous system (CNS). The CNS activity was computed on −2 (inactive) to +2 (active) scales which show all the molecules are displayed within acceptable range.

The interpretation of test compounds with reference molecule (Doxorubicin) shows that the compounds **4a–c, 5a–c, 6a–c, 7a–c, and 8a–c** were in good acceptable range and hence, they can be used to make an oral formulation for absorption and to carry by transport proteins and metabolizing

Table 2. LD_{50} ADME-TOX parameters and probability of health effects of substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylate using ACD/I-Lab 2.0

Ligand	ADME-TOX parameters									
	Intra-peritoneal[a]	Oral[a]	Intra-venous[a]	Subcu-taneous[a]	Blood effect[b]	Cardio-vascular[b]	System effect			
							Gastro-intestinal[b]	Kidney effect[b]	Liver effect[b]	Lungs effect[b]
3b	350 (0.47)	1300 (0.51)	140 (0.46)	510 (0.66)	0.97	0.56	0.84	0.68	0.43	0.96
3c	380 (0.45)	1100 (0.49)	150 (0.45)	800 (0.58)	0.97	0.55	0.57	0.55	0.43	0.98
4a	490 (0.45)	540 (0.15)	55 (0.49)	490 (0.28)	0.91	0.70	0.41	0.53	0.52	0.98
4b	640 (0.46)	460 (0.15)	58 (0.47)	940 (0.34)	0.92	0.9	0.38	0.56	0.55	0.98
4c	500 (0.4)	450 (0.18)	56 (0.48)	550 (0.33)	0.92	0.80	0.35	0.63	0.52	0.98
5a	500 (0.44)	560 (0.16)	51 (0.49)	480 (0.28)	0.90	0.75	0.39	0.57	0.51	0.97
5b	630 (0.47)	470 (0.16)	52 (0.47)	920 (0.34)	0.92	0.9	0.37	0.6	0.55	0.97
5c	520 (0.44)	450 (0.18)	52 (0.48)	550 (0.35)	0.92	0.8	0.5	0.69	0.53	0.93
6a	490 (0.45)	570 (0.16)	45 (0.51)	460 (0.29)	0.91	0.79	0.38	0.65	0.54	0.97
6b	630 (0.46)	490 (0.16)	49 (0.48)	990 (0.32)	0.93	0.91	0.36	0.74	0.57	0.94
6c	520 (0.44)	460 (0.19)	45 (0.49)	550 (0.38)	0.95	0.84	0.48	0.78	0.54	0.90
7a	490 (0.45)	580 (0.16)	42 (0.51)	450 (0.29)	0.87	0.79	0.37	0.65	0.53	0.93
7b	620 (0.47)	500 (0.16)	45 (0.48)	970 (0.31)	0.93	0.9	0.41	0.74	0.56	0.94
7c	490 (0.40)	470 (0.19)	42 (0.48)	550 (0.38)	0.90	0.84	0.28	0.78	0.52	0.93
8a	450 (0.46)	530 (0.16)	43 (0.51)	450 (0.29)	0.90	0.75	0.32	0.62	0.58	0.97
8b	580 (0.47)	460 (0.16)	46 (0.47)	850 (0.36)	0.94	0.9	0.39	0.65	0.6	0.97
8c	490 (0.4)	430 (0.19)	44 (0.48)	520 (0.35)	0.91	0.80	0.21	0.71	0.58	0.93

[a]Estimated LD_{50}-mouse value in mg/kg after Intraperitoneal, Oral, Intravenous and Subcutaneous administration.

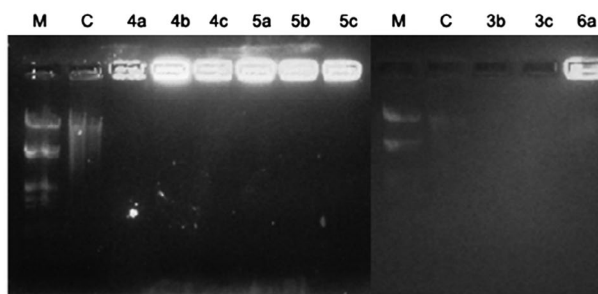
[b]Estimate of probability of blood, gastrointestinal system, kidney, liver and lung effect at therapeutic dose range of compounds (substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylate). The drugs with moderate effect on reliability index (>0.5). The drugs with border line effect on reliability index (>0.3, <0.5).

by the enzymes to maintain homeostatic condition of CNS by separation of blood/brain barrier. The intestinal absorption (log_{HIA}) and Caco-2 cell permeability (PCaco-2) within the range of −2 poor absorption and +2 more absorption show that the compounds are more permeable in intestine and help in good transport of drug metabolic compounds.

The logPGI (substrate) and non-inhibitors have drug–drug interaction within tissue that transforms xenobiotics of vigorous reduction drug absorption and released more bile (liver) and urine (kidney) (Vitali, Carroll, Chaudhry, & Hackert, 1999).

The reference range of −5 (poor) to +1 (good) and substrate inhibitor from 0 to 1 in which the reference and test compounds **4a–c, 5a–c, 6a–c, 7a–c, and 8a–c** shows good activity with human intestinal absorption and metabolism. The aqueous solubility of compounds within the range of 0 (poor)–2 (good) showed that all the molecules had good solubility, while the reference compound as well as test compounds came within acceptable range (Table 3).

The toxicity of the substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylate was predicted based on lethal dosage and functional ranges in different tissues. The LD_{50} mouse and probability of health effects were predicted using ACD/I-Lab 2.0 (guest). The toxicity of selected compounds was listed in Table 2. The LD_{50} of potential compounds detects the cumulative potential of acute toxicity administered through oral, subcutaneous, intraperitoneal, intravenous, and subcutaneous on mouse



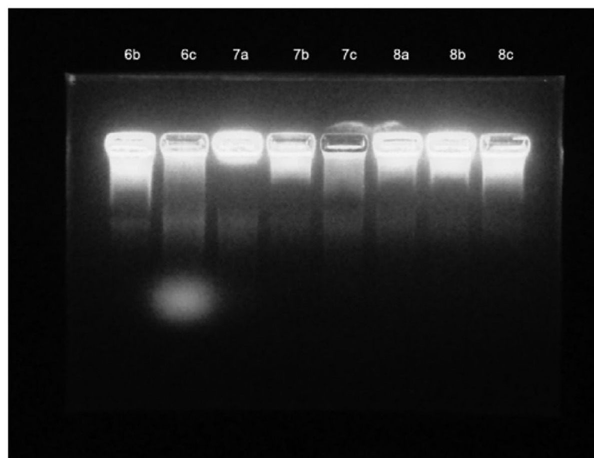


Figure 1. Photograph showing DNA cleavage on agarose gel electrophoresis by the different derivatives of the synthesized compounds.

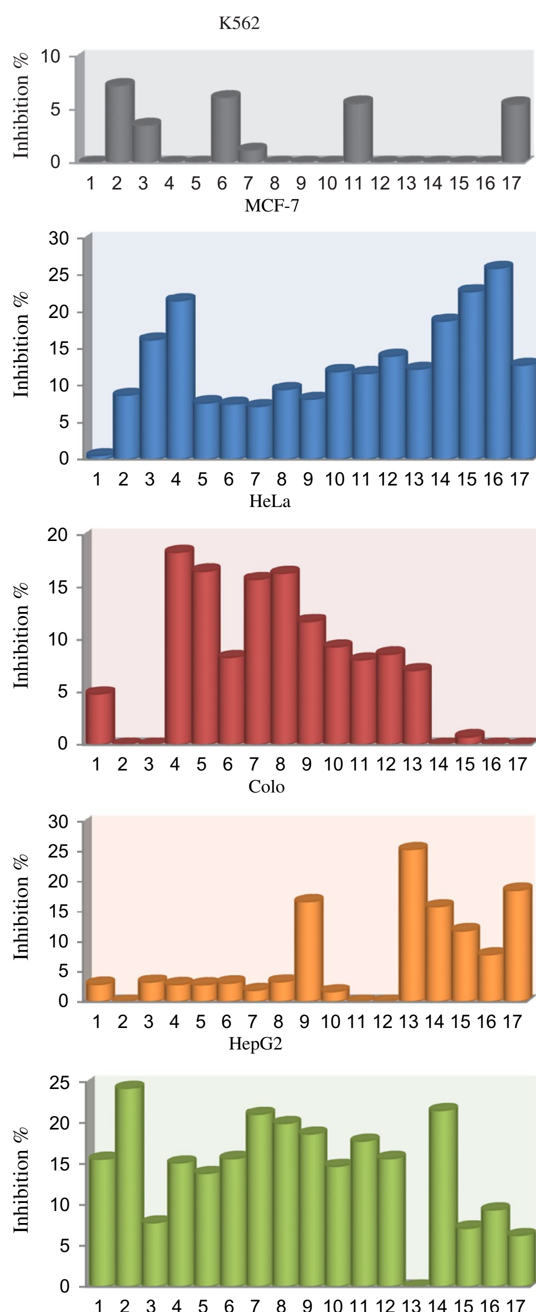
Note: In the photograph M- Standard DNA molecular weight marker (λ DNA E. Coli RI/HindIII double digest, Merck, Bangalore) **C**- Control DNA (untreated sample).

models. The comparative analysis of reference compounds with test compounds on oral, subcutaneous, intraperitoneal, and intravenous is lower when compared to reference molecule. The toxicity results suggest that the compounds **4a–c, 5a–c, 6a–c, 7a–c, and 8a–c** have less toxic effect on internal tissue and with no side effect (Table 2). Further the toxicity was tested with different organs to check the adverse effects on organs and their systems (blood, cardiovascular system, gastrointestinal system, kidney, liver, and lungs) within the therapeutic dose range. The probability of health effects revealed that the substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylate are less toxic on blood, cardiovascular system, gastrointestinal system, kidney, liver, and lungs, respectively.

4.3. DNA cleavage studies

DNA has been target for drug as it regulates many biochemical reactions which occur in the cellular system. Literature studies reveal that the clinical efficacies of many drugs correlate with their ability to induce enzyme-mediated DNA cleavage. The loci present in the DNA are involved in various regulatory processes such as gene expression, gene transcription, mutagenesis, carcinogenesis, etc. (Sreelatha, Padma, & Umadevi, 2009). In particular, designing of the compound having ability to cleave DNA is utmost important not only from the primary biological point of view but also in terms of photodynamic therapeutic approach to develop potent drugs (Kumar et al., 2014). The compounds which were found to be active in CT-DNA cleavage were screened for their antiproliferative study. The DNA cleavage of benzofuran quinoline ester hybrids were studied by agarose gel electrophoresis method, presented in Figure 1. The cleavage potential of the test compounds were examined by comparing the bands appeared in control and test compounds at 100 μg concentration. The photograph (Figure 1) clearly demonstrates that the compounds (3b–6a and 7b–8c) cleaved the DNA completely, as no traces of DNA fragments were found. The compounds (6b, 6c and 7a) have appeared to cleave DNA partially. However, the ability of reactive intermediates involved in the DNA cleavage by the compounds has not been clear. Control experiment does not reveal any significant cleavage of DNA even after prolonged exposure of the substrate.

Figure 2. Antiproliferative activity of title compounds against (a) Chronic Myelogenous Leukaemia-K562 cell line; (b) Breast Cancer-MCF-7 cell line; (c) Cervical Cancer-HeLa cell line; (d) Colorectal Adinocarcinoma-Colo cell line; (e) Hepato cellular carcinoma-HePG2 cell line; (f) Normal human kidney embryonic cell line- HEK 293.



4.4. Evaluation of antiproliferative activity

In order to evaluate with *In vitro* antiproliferative effect of the synthesized compounds was evaluated against a panel of five different human tumor cells and normal cell line, Myelogenous Leukemia (K562), Breast Cancer (MCF-7), Colorectal Adino carcinoma (Colo 205), Cervical Cancer (HeLa), Hepato cellular carcinoma (HePG2), Hepato cellular carcinoma (HePG2) and the chemotherapeutic positive control drug, Doxorubicin (Table 4). The assay was carried by dissolving the samples in dimethyl sulfoxide (DMSO; not exceeding the final concentration of 0.01%) and further diluted in cell culture medium. The antiproliferative response of synthesized molecule was assessed by MTT assay (Mosmann, 1983). The inhibition results of antiproliferative activity by different derivatives on six different cell lines are as follows: Chronic Myelogenous Leukemia (K562) was inhibited 7.27% by fluoro cinchonic ethyl carboxylate (3b) and other derivatives (Table 5) in the range of 1.22–6.16% as

Table 3. ADMET and pharmacological parameters prediction for the ligands substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylate using ADMET/SAR

Ligand	PlogBB[a]	PCaco[b]	logHIA[c]	logpGI (Non-substrate)[d]	logpGI (Non-inhibitor)[e]	PlogS[f]	logpapp[g]
3b	0.9633	0.5000	1.0000	0.7957	0.9595	−4.6654	0.9913
3c	0.9712	0.5000	1.0000	0.7810	0.9225	−4.2095	1.0112
4a	0.9430	0.5416	1.0000	0.7113	0.9010	−3.4558	1.0868
4b	0.9709	0.5732	1.0000	0.7055	0.7329	−3.9795	1.1292
4c	0.9656	0.5853	1.0000	0.7231	0.8397	−4.3351	1.1113
5a	0.9590	0.5368	1.0000	0.8200	0.8898	−3.8367	1.0243
5b	0.9786	0.5627	1.0000	0.6647	0.6760	−4.3649	1.0763
5c	0.9711	0.5757	1.0000	0.6834	0.7854	−4.7044	1.0569
6a	0.9561	0.5441	1.0000	0.8241	0.9279	−3.8777	1.1000
6b	0.9756	0.5488	1.0000	0.6299	0.5971	−4.3153	1.1030
6c	0.9672	0.5597	1.0000	0.6471	0.6952	−4.5983	1.0871
7a	0.9555	0.5701	1.0000	0.8113	0.9439	−4.1205	1.1339
7b	0.9731	0.5656	1.0000	0.5587	0.5911	−4.4577	1.1315
7c	0.9648	0.5764	1.0000	0.8175	0.9394	−4.7087	1.1182
8a	0.9476	0.5000	1.0000	0.8182	0.8790	−3.9499	1.0209
8b	0.9753	0.5359	1.0000	0.6772	0.5905	−4.4093	1.0611
8c	0.9692	0.5480	1.0000	0.8256	0.9386	−4.7017	1.0470
Doxoru-bicin	0.9898	0.8257	0.6345	0.8824	0.9306	−2.9266	−0.6751

[a]Predicted blood/brain barrier partion coefficient (1-high penetration, 2- medium penetration and 3- low penetration).
[b]Predicted Caco-2 cell permeability in nm/s (acceptable range −1 is poor, + 1 is great).
[c]Predicted human intestinal absorption in nm/s (acceptable range 0 is poor, > 1 is great).
[d]Predicted P-glycoprotein Substrate in nm/s (acceptable range of −5 is poor, 1 is great).
[e]Predicted P-glycoprotein inhibitor in nm/s (acceptable range 0–1).
[f]Predicted aqueous solubility, (Concern value is 0–2 highly soluble).
[g]Predicted Caco-2 cell Permeability in cm/s (Concern value is −1 to 1).

Table 4. Inhibition of standard drug Doxorubicin on the various cell lines employed for the study

Cancer cell lines	Avg % in & SD of cell lines	Doxorubicin Conc. 1uM
K562	Avg % in	95.57
	SD	2.2256
MCF-7	Avg % in	97.61
	SD	2.189
Hela	Avg % in	97.16
	SD	2.27
Colo	Avg % in	91.55
	SD	1.8723
HepG2	Avg % in	97.355
	SD	1.5699
HEK	Avg % in	5.678
	SD	1.56

Table 5. Particulars of the derivatives of 2-(1-benzofuran-2-yl) quinoline-4-carboxylate

Sl. No.	Samples code	R	R_1	Molecular formula	Molecular weight	(%)Yield	Melting point (°C)
1	3b	H	F	$C_{18}H_{10}FNO_3$	307.27	85	295–298
2	3c	Cl	H	$C_{18}H_{11}ClNO_3$	323.72	85	308–310
3	4a	H	H	$C_{19}H_{13}NO_3$	303.31	83	115–118
4	4b	H	F	$C_{19}H_{12}FNO_3$	301.30	80	122–12
5	4c	Cl	H	$C_{19}H_{12}ClNO_3$	337.75	85	125–128
6	5a	H	H	$C_{20}H_{15}NO_3$	371.33	84	68–70
7	5b	H	F	$C_{20}H_{14}FNO_3$	335.32	80	80–82
8	5c	Cl	H	$C_{20}H_{14}ClNO_3$	351.78	85	84–85
9	6a	H	F	$C_{22}H_{18}FNO_3$	363.38	82	88–90
10	6b	H	H	$C_{22}H_{19}NO_3$	345.39	87	76–78
11	6c	Cl	H	$C_{22}H_{18}ClNO_3$	379.832	84	92–94
12	7a	H	H	$C_{21}H_{17}NO_3$	331.36	79	78–80
13	7b	H	F	$C_{21}H_{16}FNO_3$	349.35	83	122–123
14	7c	Cl	H	$C_{21}H_{16}ClNO_3$	365.80	82	133–134
15	8a	H	H	$C_{19}H_{13}NO_3$	331.36	76	79–80
16	8b	H	F	$C_{19}H_{12}FNO_3$	349.35	81	118–120
17	8c	Cl	H	$C_{19}H_{12}ClNO_3$	365.80	74	129–130

shown in Figure 2(a); Breast Cancer (MCF-7) cell line was inhibited 25.97% by fluoro cinchonic isopropyl carboxylate (8b) and remaining derivatives in the range of 7.24–22.82% as showed in Figure 2(b); Cervical Cancer (HeLa) cell line was inhibited 18.35% by fluoro cinchonic methyl carboxylate (4b) and other derivatives in the range of 4.89–16.57% as shown in Figure 2(c); Colorectal Adeno carcinoma (Colo 205) was inhibited 25.36% by fluoro cinchonic n-butyl carboxylate (7a) and remaining derivatives in the range of 2.85–18.61% as shown in Figure 2(d); Hepato cellular carcinoma (HePG2) was inhibited 24.22% by fluoro cinchonic acid (7b) and other derivatives in the range of 7.17–21.52% as shown in Figure 2(e). The results showed that of all the different derivatives, fluoro-substituted carboxylate has reasonable inhibition on different cancer cell lines.

5. Conclusion

The cinchophen analogs are known for their varied medicinal properties and reported for various biological activities. In the present study, an attempt is made to predict ADMET/SAR *in silico*; the synthesized molecules in acceptable range were further investigated for antiproliferative activity on different human cancer cell lines, apart from their DNA cleavage study. The present work indicated that the benzofuran quinoline esters were found to possess significant antiproliferative and potent DNA cleavage activity.

The potent esters will be further taken up for in-depth study by varying the functionalities on the aromatic rings with various electrophiles and nucleophiles, with reference to the commercially available anticancer drug Doxorubicin. Further examination is required for the modification of lead to increase the antiproliferative potency.

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