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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, anti-microbial and anti-cancer evaluation study of 3-(3-benzofuranyl)-coumarin derivatives

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Abstract The series of 3-coumarin-substituted benzofuran derivatives **4a–4j** have been synthesized under optimized experimental condition with excellent yields. All the isolated compounds were characterized and screened anti-microbiological and anti-cancer activity. The anti-microbiological results observed were extremely good against *S. aureus*, *C. albicans* and *A. niger*. The comparative docking studies with gyrase type IIA topoisomerase from *mycobacterium tuberculosis* docked with ligands and **4j** have found lowest docked energy.

Keywords 4-Bromomethylcoumarin · Benzofuran-3-acetic acid · 3-(3-Benzofuranyl)-coumarin · Anti-microbial activity and anti-cancer activity

Introduction

The benzofuran derivatives are present in natural products, which are exhibited excellent biological activities and play an important role in both drug discovery and chemical biology (Cragg *et al*., 1997; Shu, 1998). Benzofuran derivatives shows broad range of activities, substitution of aryl or hetero aryl system at C2 position of benzofuran nucleus exhibited excellent anti-microbiological activity at low concentration (Koca *et al*., 2005; Kirilmis *et al*., 2008; Bondock *et al*., 2011; Jiang *et al*., 2011; Liu *et al*., 2012; Rajanarendar *et al*., 2013; Khan *et al*., 2005; Fuganti and Serra, 1998). Recently, the research on benzofuran derivatives shows versatile biological activities such anti-inflammatory, analgesic (Fuganti and Serra, 1998), anti-cancer (Parekh *et al*., 2011), anti-oxidant (Karatas *et al*., 2006) and anti-tubercular activities (Manna and Agrawal, 2010, 2011). Benzofuran derivatives also exhibit CYP19 inhibitory activity (Saberi *et al*., 2006), and cytotoxicity properties were reported (Saberi *et al*., 2006; Bigler *et al*., 2007). On the other hand, coumarin and its derivatives have been recognized as an anti-inflammatory (Pochet *et al*., 1996), anti-microbial (Yu *et al*., 2003), antiviral (Evstropov *et al*., 1992), anti-tumour (Belluti *et al*., 2010) and anti-coagulant activities (Manolov and Danchev, 1995). The coumarin derivatives are also known as fluorescent brightening agents (Corrie *et al*., 2000) and a dying agent (Sun *et al*., 1998). Therefore, the recent investigations of 3-arylcoumarins derivatives are selective monoamine oxidase B inhibitors (Wang *et al*., 2011) and HIV-1 replication inhibitors (Olmedo *et al*., 2012). The literature reports reveal that coumarin derivatives are exhibited broad range of biological activities (Singhapol *et al*., 2013; China *et al*., 2010; Quezada *et al*., 2010). Whereas number of studies describes the anti-cancer activity mechanism of coumarin derivatives and a bioactive coumarin derivative, Osthole is extracted from *Cnidium monnieri* (*L*) cusson which is more effective against human hepatocellular carcinoma (HHC) (Xu *et al*., 2011; Vazquez *et al*., 2012; Avin *et al*., 2014). Similarly, coumarin monastrol hydride also performs anti-cancer activity against many cancer cell lines associated by apoptotic activity (Sashidhara *et al*.,

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2013), whereas the pyrazoline-substituted coumarin nucleus received considerable chemotherapeutic potential and are found to be good cytotoxic activity (Amin *et al*., 2015). In connection with our previously reported 4-substituted coumarin derivatives showed interesting biological activities, such as anti-microbial, analgesic and anti-fungal activities (Shastri *et al*., 2004). Therefore, the importance and significance of the structural information of both heterocycles, it was thought of considerable interest to synthesis 3-benzofuran-substituted coumarin derivatives which is represented in the Scheme 1. The synthesized compounds (**4a**–**4j**) were screened in vitro microbiological and anti-cancer activity.

Experimental

Materials and methods

The melting points were determined by open capillary method and are uncorrected. IR spectra (KBr disc) were recorded on Nicolet 5700 FT-IR spectrometer. NMR spectra were recorded on Bruker 400 MHz Spectrometer using DMSO-d_6 as solvents and TMS as internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were recorded using Shimadzu GCMS-QP2010S. TLC was performed on silica gel G for TLC (Merck), and spots were visualized by iodine vapour or by irradiation with UV light (254 nm) and also purity of the compound was checked by TLC. All the chemicals purchased were of analytical grade and were used without further purification.

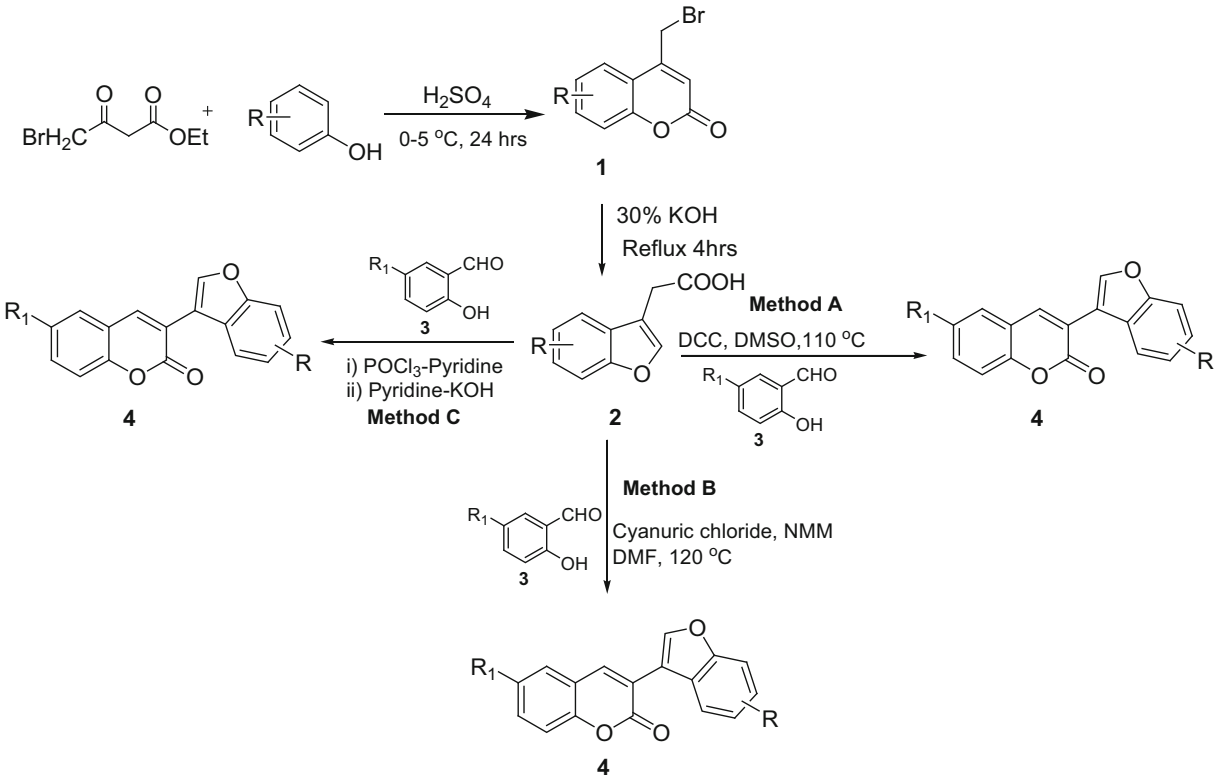
General procedures for synthesis of 3-(3-benzofuranyl)-coumarins (4a–4j)

Method (A)

A solution of substituted salicylaldehyde (**3**) (0.8 mmol) and the substituted benzofuran-3-acetic acid (**2**) (1.0 mmol) in DMSO (15 mL) was prepared. DCC (2.0 mmol) was added, and the mixture was heated on oil bath at 110 °C for about 8–10 h. The completion of the reaction was confirmed by TLC. Reaction mixture was allowed to cool and poured into ice-cold water with stirring. The separated solid was filtered and washed with water. The crude product was purified by recrystallization from ethanol to afford 3-(3-benzofuranyl)-coumarin derivatives (**4**).

Method (B)

A mixture of cyanuric chloride (1.0 mmol), NMM (1.5 mmol), and the substituted benzofuran-3-acetic acid



Scheme 1 R = 5-Me; 6-Me; 5-OMe; 4,5-Benzo; 6,7-Benzo; R_1 = H & Br

(1.0 mmol) in DMF (5 mL) was stirred at room temperature for 10 min. After this substituted salicylaldehyde (0.8 mmol) was added. Subsequently, the resulting reaction mixture was refluxed for about 4–5 h. Completion of the reaction was monitored by TLC. The reaction mixture was diluted with water (10 mL) and extracted three times with EtOAc (20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography-9:1 (Hexane: EtOAc) (**4**).

Method (C)

A solution of substituted salicylaldehyde (0.8 mmol) and the corresponding substituted benzofuran-3-acetic acid (1.0 mmol) in pyridine were stirred at room temperature and a solution of $POCl_3$ (1.0 mmol) in ether was added to it over 30 min. The reaction mixture was stirred for 1 h and diluted with ice-cold HCl. The solid product thus formed was isolated and washed with cold water, 10 % $NaHCO_3$, and again with water and then crystallized by ethanol. The solution of ester in pyridine was added to pulverized potassium hydroxide (KOH) in pyridine in 30 min. The reaction mixture was stirred for 30–60 min and then diluted with HCl. The solid product obtained was washed with water, dried, and crystallized by ethanol (**4**).

3-(5-Methyl-3-benzofuranyl)-coumarin (**4a**) White coloured solid, m.p. 139–141 °C; Yield 86 %; IR (KBr): 1728 (C=O stretching) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 8.58 (s, 1H, C2-H of benzofuran), 8.56 (*s*, 1H, C4-H of coumarin), 7.96 (*d*, 1H, $J = 7.6$ Hz, C5-H of coumarin), 7.93 (*s*, 1H, C4-H of benzofuran), 7.65 (*t*, 1H, $J = 7.2$ Hz, C7-H of coumarin), 7.55 (*d*, 1H, $J = 8.4$ Hz, C8-H of coumarin), 7.46 (*d*, 1H, $J = 8.4$ Hz, C7-H of benzofuran), 7.39 (*t*, 1H, $J = 7.6$ Hz, C6-H of coumarin), 7.23 (*dd*, 1H, $J = 8.4$ Hz, $J = 2.5$ Hz, C6-H of benzofuran), 2.49 (*s*, 3H, Ar–CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 159.02, 152.89, 152.08, 146.50, 138.00, 132.61, 131.54, 128.72, 126.18, 124.99, 124.60, 120.93, 119.21, 119.13, 115.85, 114.08, 111.24, 21.05; GC–MS (*m/z*): 276.

3-(6-Methyl-3-benzofurany)-coumarin (**4b**) White coloured solid, m.p. 172–174 °C; Yield 89 %; IR (KBr): 1716 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.56 (*s*, 1H, C2-H of benzofuran), 8.22 (*s*, 1H, C4-H of coumarin), 7.80 (*d*, 1H, $J = 8.0$ Hz, C5-H of coumarin), 7.63 (*d*, 1H, $J = 7.5$ Hz, C8-H of coumarin), 7.56 (*t*, 1H, $J = 7.5$ Hz, C7-H of coumarin), 7.42 (*d*, 1H, $J = 7.5$ Hz, C4-H of benzofuran), 7.43 (*s*, 1H, C7-H of benzofuran), 7.36 (*t*, 1H, $J = 7.5$ Hz, C6-H of coumarin), 7.22 (*dd*, 1H, $J = 8.0$ Hz, $J = 2.4$ Hz, C5-H of benzofuran), 2.50 (*s*, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.01, 152.87, 152.07, 147.46, 137.91, 132.59, 128.71, 126.30, 126.03, 124.97, 124.67, 124.52, 119.19, 119.11, 115.89, 114.07, 111.24, 21.19; GC–MS (*m/z*): 276.

3-(5-Methoxy-3-benzofuranyl)-coumarin (**4c**) Grey coloured solid, m.p. 120–122 °C; Yield 82 %; IR (KBr): 1716 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.56 (*s*, 1H, C2-H of benzofuran), 8.53 (*s*, 1H, C4-H of coumarin), 7.97 (*dd*, 1H, $J = 8.0$ Hz, $J = 1.6$ Hz, C5-H of coumarin), 7.65 (*d*, 1H, $J = 7.2$ Hz, C8-H of coumarin), 7.61 (*t*, 1H, $J = 7.5$ Hz, C7-H of coumarin), 7.54 (*d*, 1H, $J = 2.4$ Hz, C4-H of benzofuran), 7.47 (*d*, 1H, $J = 7.2$ Hz, C7-H of benzofuran), 7.41 (*t*, 1H, $J = 7.6$ Hz, C6-H of coumarin), 7.03 (*dd*, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz, C6-H of benzofuran), 3.86 (*s*, 3H, Ar-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.04, 156.06, 152.14, 149.34, 147.28, 146.92, 138.41, 131.55, 128.78, 125.66, 124.69, 119.20, 115.91, 114.52, 113.15, 112.91, 104.57, 55.98; GC–MS (*m/z*): 292.

3-(naphtho[2,1-b]-2-furanyl)-coumarin (**4d**) Light brown solid, m.p. 212–214 °C; Yield 79 %; IR (KBr): 1716 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.35 (*s*, 1H, C2-H of benzofuran), 8.28 (*s*, 1H, C4-H of coumarin), 8.06 (*t*, 1H, $J = 5.2$ Hz, C5-H of coumarin), 7.93 (*d*, 1H, $J = 9.2$ Hz, C8-H, of coumarin), 7.87 (*d*, 1H, $J = 8.4$ Hz, C9-H, of naphtho), 7.82 (*dd*, 2H, $J = 8.0$ Hz, $J = 1.6$ Hz, C4-H & C7-H of naphtho), 7.73 (*t*, 1H, $J = 7.2$ Hz, C6-H of coumarin), 7.55 (*d*, 1H, $J = 8.4$ Hz, C8-H of naphtho), 7.50 (*t*, 2H, $J = 7.2$ Hz, C5-H & C6-H of naphtho), 7.44 (*t*, 1H, $J = 7.6$ Hz, C7-H of coumarin); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.71, 153.51, 152.39, 144.40, 143.10, 132.13, 130.36, 128.74, 128.60, 127.39, 126.49, 126.16, 124.76, 124.57, 123.97, 123.48, 120.50, 119.00, 117.51, 116.23, 112.55; GC–MS (*m/z*): 312.

3-(naphtho[2,1-b]-1-furanyl)-coumarin (**4e**) Light brown coloured solid, m.p. 184–186 °C; Yield 81 %; IR (KBr): 1717 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.74 (*s*, 1H, C2-H of benzofuran), 8.65 (*s*, 1H, C4-H of coumarin), 8.31 (*d*, 1H, $J = 8.4$ Hz, C5-H of coumarin), 8.20 (*d*, 1H, $J = 8.4$ Hz, C8-H of coumarin), 8.10 (*d*, 1H, $J = 8.0$ Hz, C4-H of naphtho), 7.98 (*dd*, 1H, $J = 7.6$ Hz, $J = 1.6$ Hz, C9-H of naphtho), 7.90 (*d*, 1H, $J = 8.4$ Hz, C5-H of naphtho), 7.71 (*t*, 1H, $J = 6.8$ Hz, C6-H of coumarin), 7.62 (*t*, 2H, $J = 8.8$ Hz, C7-H & C8-H of naphtho), 7.49 (*d*, 1H, $J = 8.4$ Hz, C6-H of naphtho), 7.42 (*t*, 1H, $J = 7.6$ Hz, C7-H of coumarin); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.06, 152.27, 149.88, 145.45, 138.68, 131.65, 130.97, 128.74, 128.28, 126.97, 125.83, 124.63, 123.83, 120.80, 120.47, 119.55, 119.48, 119.18, 119.03, 115.86, 115.78; GC–MS (*m/z*): 312.

6-Bromo-3-(5-methyl-3-benzofuranyl)-coumarin (**4f**) White coloured solid, m.p. 170–172 °C; Yield 84 %; IR (KBr): 1724 (C=O stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz)

δ: 8.61 (*s*, 1H, C2-H of benzofuran), 8.56 (*s*, 1H, C4-H of coumarin), 8.25 (*d*, 1H, $J = 2.4$ Hz, C5-H of coumarin), 7.99 (*s*, 1H, C4-H of benzofuran), 7.76 (*dd*, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz, C7-H of coumarin), 7.57 (*d*, 1H, $J = 8.4$ Hz, C8-H of coumarin), 7.43 (*d*, 1H, $J = 8.8$ Hz, C7-H of benzofuran), 7.24 (*dd*, 1H, $J = 8.4$ Hz, $J = 1.2$ Hz, C6-H of benzofuran), 2.48 (*s*, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.51, 152.85, 151.00, 146.80, 145.93, 136.41, 133.70, 132.75, 130.84, 126.38, 124.71, 121.12, 120.91, 118.18, 116.14, 113.73, 111.28, 21.17; GC–MS (*m/z*): 354.

6-Bromo-3-(6-methyl-3-benzofuranyl)-coumarin (**4g**) White coloured solid, m.p. 206–208 °C; Yield 85 %; IR (KBr): 1726 (C=O stretching) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.58 (*s*, 1H, C2-H of benzofuran), 8.47 (*s*, 1H, C4-H of coumarin), 8.23 (*s*, 1H, C5-H of coumarin), 7.74 (*dd*, 1H, $J = 8.4$ Hz, $J = 2.2$ Hz, C7-H of coumarin), 7.69 (*d*, 1H, $J = 8.0$ Hz, C8-H of coumarin), 7.56 (*s*, 1H, C7-H of benzofuran), 7.34 (*d*, 1H, $J = 6.2$ Hz, C4-H of benzofuran), 7.27 (*dd*, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz, C5-H of benzofuran), 2.49 (*s*, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.57, 152.91, 151.07, 147.43, 145.84, 136.38, 133.04, 130.32, 130.07, 126.36, 124.78, 124.49, 120.88, 118.14, 116.17, 113.75, 111.27, 21.29; GC–MS (*m/z*): 354.

6-Bromo-3-(5-methoxy-3-benzofuranyl)-coumarin (**4h**) Grey coloured solid, m.p. 138–140 °C; Yield 87 %; IR (KBr): 1731 (C=O stretching) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.58 (*s*, 1H, C2-H of benzofuran), 8.47 (*s*, 1H, C4-H of coumarin), 8.23 (*d*, 1H, $J = 2.4$ Hz, C5-H of coumarin), 7.75 (*dd*, 1H, J = 8.8 Hz, $J = 2.4$ Hz, C7-H of coumarin), 7.59 (*d*, 1H, $J = 8.8$ Hz, C8-H of coumarin), 7.56 (*d*, 1H, $J = 2.4$ Hz, C4-H of benzofuran), 7.41 (*d*, 1H, $J = 8.8$ Hz, C7-H of benzofuran), 7.02 (*dd*, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz, C6-H of benzofuran), 3.86 (*s*, 3H, Ar-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 148.50, 148.97, 138.86, 137.79, 134.63, 134.56, 131.77, 131.16, 119.81, 118.79, 118.17, 113.93, 113.21, 112.79, 112.13, 105.87, 105.24, 58.50; GC–MS (*m/z*): 370.

6-Bromo-3-(naphtho[2,1-b]-2-furanyl)-coumarin (**4i**) Light brown coloured solid, m.p. 173–175 °C; Yield 77 %; IR (KBr): 1723 (C=O stretching) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.30 (*s*, 1H, C2-H of benzofuran), 8.28 (*s*, 1H, C4-H of coumarin), 8.08 (*d*, 1H, $J = 2.4$ C5-H of coumarin), 8.04 (*d*, 1H, $J = 5.2$ Hz, C8-H of coumarin), 7.93 (*d*, 1H, $J = 9.2$ C9-H of naphtho), 7.89 (*d*, 1H, $J = 6.4$ Hz, C4-H of naphtho), 7.87 (*d*, 1H, $J = 6.5$ Hz, C7-H of naphtho), 7.85 (*dd*, 1H, $J = 6.4$ Hz, $J = 2.4$ Hz, C7-H of coumarin), 7.53 (*d*, 1H, $J = 5.6$ Hz, C8-H of naphtho), 7.51–7.47 (*m*, 2H, C5-H & C6-H of naphtho); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.26, 152.59, 152.43, 144.78, 144.34, 141.89, 141.50, 134.41, 130.61, 128.77, 127.31, 126.50, 126.30, 124.73, 123.63, 121.33, 120.92, 118.50, 117.22, 116.27, 112.55; GC–MS (*m/z*): 390.

6-Bromo-3-(naphtho[2,1-b]-1-furanyl)-coumarin (**4j**) Light brown coloured solid, m.p. 196–198 °C; Yield 80 %; IR (KBr): 1734 (C=O stretching) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.78 (*s*, 1H, C2-H of benzofuran), 8.65 (*s*, 1H, C4-H of coumarin), 8.31 (*d*, 1H, $J = 8.0$ Hz, C8-H of coumarin), 8.26 (*d*, 1H, $J = 2.0$ Hz, C5-H of coumarin), 8.25 (*d*, 1H, $J = 7.2$ Hz, C4-H of naphtho), 8.12 (*d*, 1H, $J = 8.4$ Hz, C9-H of naphtho), 7.92 (*d*, 1H, $J = 8.8$ Hz, C5-H of naphtho), 7.78 (*dd*, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz, C7-H of coumarin), 7.70 (*t*, 1H, $J = 7.2$ Hz, C8-H of naphtho), 7.61 (*t*, 1H, $J = 8.4$ Hz, C7-H of naphtho), 7.46 (*d*, 1H, $J = 8.8$ Hz C6-H of naphtho); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.03, 152.22, 151.25, 145.85, 137.05, 133.83, 131.01, 130.76, 128.29, 127.04, 125.92, 123.95, 121.13, 120.45, 119.49, 119.43, 118.14, 116.16, 115.85, 115.68, 115.37; GC–MS (*m/z*): 390.

Anti-bacterial activity

The anti-bacterial screening of all the synthesized 3-(3-benzofuranyl) coumarin (**4a**–**4j**) derivatives was carried out against a broad range of pathogenic microbial test strains using broth dilution technique. The stock solutions of synthesized compounds were reconstituted with a minimum amount of dimethylsulphoxide (DMSO). This solvent did not possess any anti-microbial activity of its own. To evaluate the anti-microbial activities against four pathogenic bacterial strains namely two gram-positive (*S. aureus and B. subtilis*) and two gram-negative (*E. coli and Pseudomonas*) bacteria by broth dilution method.

Anti-fungal activity

Anti-fungal activity was done by broth dilution method. For assaying anti-fungal activity, *C. Albicans* and *A. niger* strain were recultured in DMSO. A close investigation of the MIC values indicated that all the compounds exhibited a varied range of MIC of anti-fungal activity against all the tested fungal strains.

Anti-cancer activity

Anti-cancer activity was done by colorimetric assay that measures the reduction in yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent

DMSO, and the released, solubilized formazan reagent is measured colorimetrically. Since reduction in MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Cell culture

The cell line was maintained in 96-well micro titre plate containing MEM media supplemented with 10 % heat inactivated foetal calf serum (FCS), containing 5 % of mixture of gentamycin, penicillin (100 Units/ml) and streptomycin (100 µg/ml) in the presence of 5 % CO_2 at 37 °C for 3–4 days. After 3–4 days, the supernatant was removed and MEM media were replaced with Hank's balanced solution supplemented with gentamycin, penicillin and streptomycin and incubate overnight.

Cytotoxicity assay

The supernatant was removed from the plate, and fresh Hank's balanced salt solution was added and treated with different concentration of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. After 24-h incubation at 37 °C in a humidified atmosphere of 5 % CO_2, the medium was replaced with MTT solution (100 µl, 5 mg per ml in MEM medium) for further 4-h incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue" were solubilized by adding DMSO (200 µl), and optical density was measured at wavelength of 492 nm.

Docking study

Docking simulations were performed with HEX software (v8.0). Dock energies were calculated based on shape and electrostatics using default grid spacing of 6.0Å.

Results and discussion

Chemistry

3-(3-Benzofuranyl) coumarin derivatives (**4**) were synthesized efficiently by the protocol outlined in the Scheme 1. The 4-bromomethylcoumarins (**1**) were prepared by Pechmann cyclization using different phenols with 4-bromoethylacetoacete in the presence of sulphuric acid as condensing agent (Kulkarni and Patil, 1981). Further, conversion of 4-bromomethyl coumarins (**1**) to benzofuran-3-acetic acid (**2**) via ring opening and ring closer by S_N2 reaction followed by aromatization (Fall *et al*., 1995) using 30 % sodium hydroxide under reflux condition (Scheme 1). The treatment of different condensing reagents with substituted salicylaldehyde (**3**) and benzofuran-3-acetic acid (**2**) achieved desired product 3-(3-benzofuranyl) coumarin (**4**). Earlier, the title compound was synthesized by Deshpande and Paradkar (Deshpande and Paradkar, 1990) using different reaction condition and reagent in several steps. We here reporting the desired product in single step by three different literature methods and represented in Scheme 1: in method A, using N,N'-dicyclohexyl-carbodiimide (DCC) as a condensing agent in the presence of dimethylsulphoxide at 110 °C (Olmedo *et al*., 2012) and in method B, cyanuric chloride in the presence of base N-methyl morpholine (NMM) at 120 °C (Sashidhara *et al*., 2012), whereas in method C, phosphorus oxychloride-pyridine at room temperature (Taksande *et al*., 2010). All the three methods afforded identical product **4** with excellent yield (>70 %). Among these methods, using cyanuric chloride with NMM is the best method to carry out the reaction because of the simple experimental procedure, easy isolation as well as purity of the product (Table 1). The mechanisms of each methods were discussed in Schemes 2, 3 and 4, and this involves in the formation of salicylaldehyde ester followed by intermolecular Knoevenagel condensation to form a coumarin skeleton.

All the synthesized 3-(3-benzofuranyl) coumarins (**4a**–**4j**) were well supported by spectroscopic analysis as well as single-crystal X-ray analysis study. In case of compound **4h** ($R = C5–OCH_3$; $R_1 = Br$), IR spectrum showed 1716 cm^{-1} is due to lactone carbonyl stretching band of coumarin. Further, the formation of compound **4h** was confirmed by ^1H NMR, two singlet at 8.58 δppm and 8.47 δppm are due to C2-H of benzofuran and C4-H of coumarin, respectively, and doublet at 8.23 δppm with a coupling constant of J = 2.4 Hz is due to C5-H of coumarin. The doublet of doublets at 7.74 δppm (J = 2.4 Hz & J = 8.8 Hz) is due to C7-H of coumarin and another doublet at 7.59 δppm (J = 8.8 Hz) is due to C8-H of coumarin, whereas doublet at 7.56 δppm (J = 2.4 Hz) is due to C4-H of benzofuran. The doublet at 7.41 δppm (J = 8.8 Hz) is due to C7-H of benzofuran, and the one more doublet of doublet at 7.02 δppm (J = 2.4 Hz & J = 8.8 Hz) is due to C6-H of benzofuran. The OCH_3 singlet was observed at 3.86 δppm. The assignment of all the protons was done by using 2D NMR (Fig. 1), and finally, the spectral data are well agreement with single-crystal analysis study of the compound **4h** (Fig. 2).

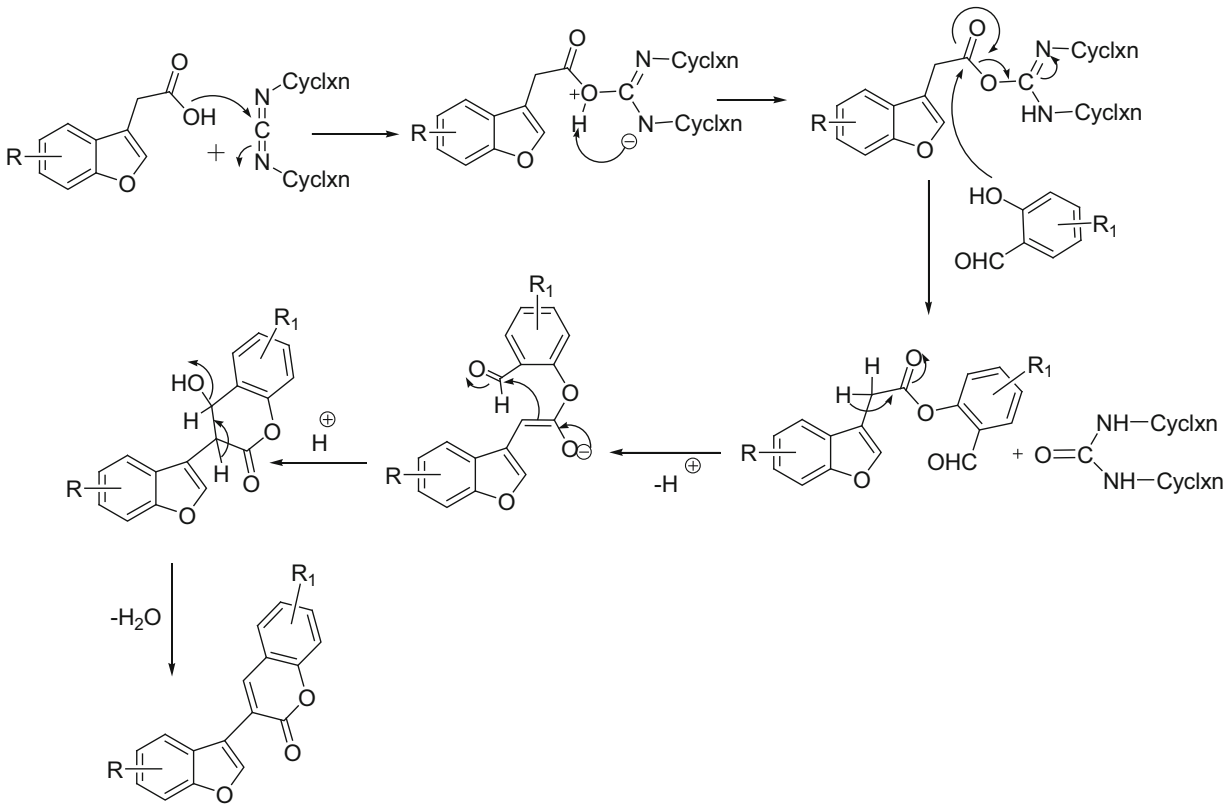
Biological screening

Anti-bacterial screening

The synthesized all the compounds were screened their in vitro anti-bacterial activity against gram-positive (*S. aureus and B. subtilis*) and gram-negative (*E. coli and*

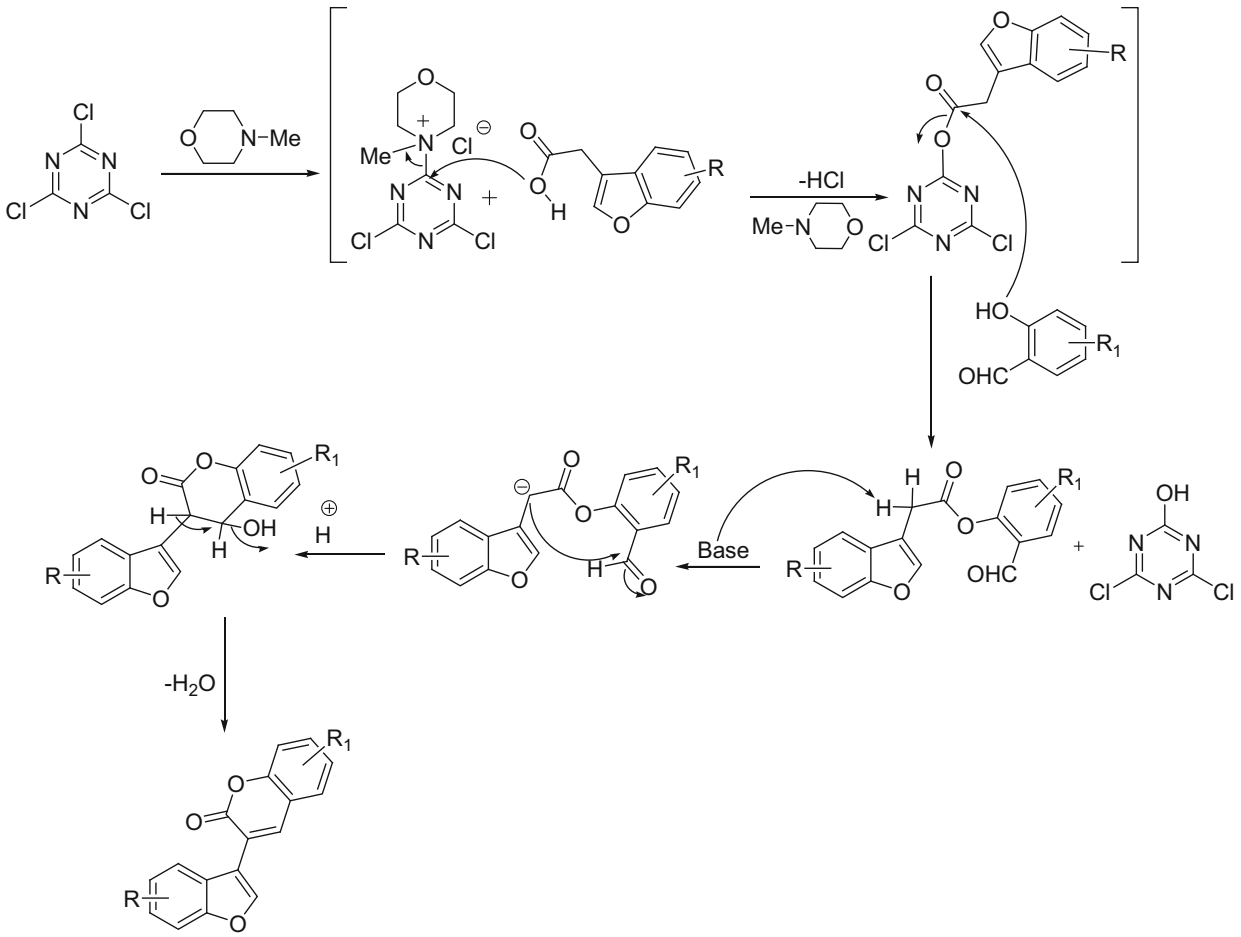
Table 1 Synthesis of compounds (**4a**–**4j**) in different methods

Compounds	R	R_1	Method A		Method B		Method C	
			Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)
4a	5-Me	H	78	8.0	86	4.0	72	3.0
4b	6-Me	H	81	8.0	89	4.0	75	3.0
4c	5-OMe	H	79	8.5	82	4.5	73	3.5
4d	4,5-Benzo	H	74	9.0	79	5.0	65	4.0
4e	6,7-Benzo	H	76	9.5	81	5.0	71	4.0
4f	5-Me	Br	79	8.5	84	4.5	73	3.5
4g	6-Me	Br	81	8.5	85	4.5	71	3.5
4h	5-OMe	Br	79	9.0	87	5.0	67	4.0
4i	4,5-Benzo	Br	72	9.5	77	5.5	63	4.5
4j	6,7-Benzo	Br	75	10.0	80	5.5	69	4.5

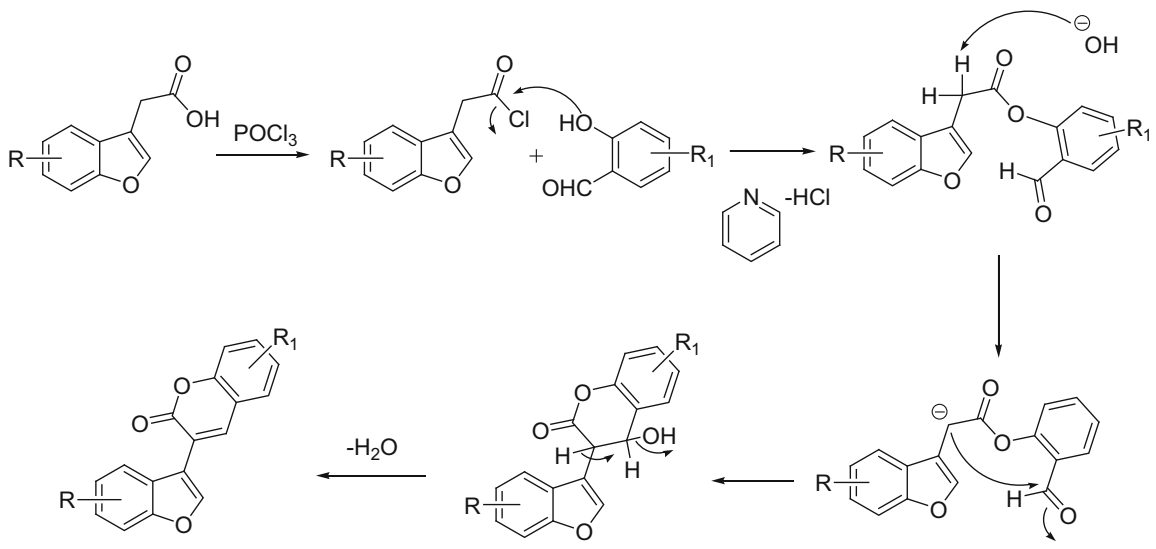


Scheme 2 Method A

Pseudomonas) bacteria by broth dilution method (Table 2). The results revealed that some of the compounds were highly active against gram-positive bacteria (*S. aureus* and *B. subtilis*). The activity against *S. aureus* compound **4a** and **4b** is methyl substitution at C5 and C6 on benzofuran nucleus shows very high activity up to 0.2 μgm/mL, and same results were observed in compounds **4i** and **4j** having substitution on both nucleus such as benzo substitution on benzofuran and bromo substitution on coumarin nucleus. Similarly, compounds **4c**, **4d**, **4f** and **4h** are exhibited very good activity, whereas **4e** and **4g** are less active compared to standard and compounds **4a**, **4b**, **4c**, **4g** and **4j** are highly active against *B. subtilis* compared to standard drug. In case of gram-negative bacteria, all the synthesized compounds were very less active, against *E. coli* and *Pseudomonas*.



Scheme 3 Method B



Scheme 4 Method

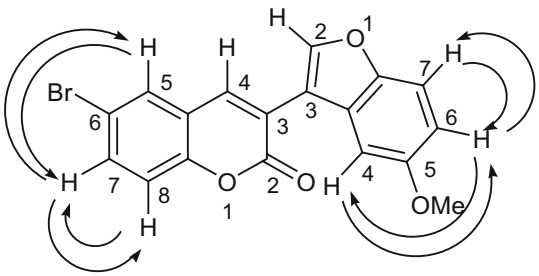


Fig. 1 Proton coupling assignment (**4h**)

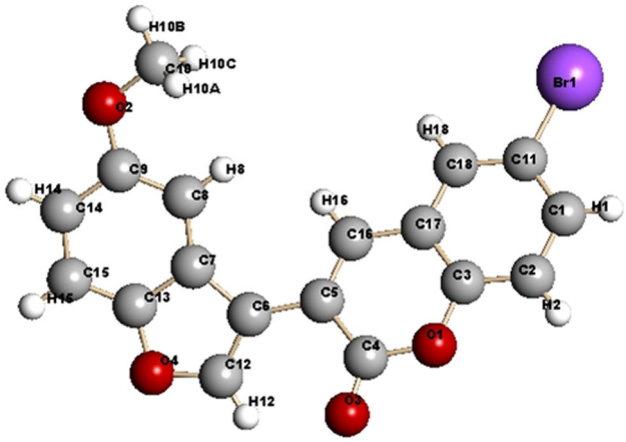


Fig. 2 ORTEP diagram of compound (**4h**)

Anti-fungal screening

The synthesized compounds **4a** to **4j** were screened for their anti-fungal activity against *C. Albicans* and *A. niger* by Broth dilution method. All the compounds exhibited excellent anti-fungal activity against *C. Albicans* and *A. niger*, and the results are summarized in Table 2.

Anti-cancer activity

The in vitro anti-cancer activity of synthesized compounds (**4a**–**4j**) was tested with *HeLa* cell lines using MTT assay, and the results are tabulated in Table 3. The growth of inhibition and effect of test compounds were assessed by calorimetric determination. Compounds **4a, 4b** and **4j** showed the anti-cancer activity towards *HeLa* cell lines with IC_{50} values 20 and 25 μg, respectively. In the observation results, rest of the compounds had no impact on the growth of cell at less than 50 μg concentration, but at higher concentration exhibited activity.

Docking studies

In the present investigation compounds obtained from synthetic approach which is potential inhibitors of gyrase type-IIA topoisomerase from *Mycobacterium tuberculosis* and new modelling study supports the new binding mode for this coumarin derivatives.

To better understand the binding mode of compounds for docking studies, 2D structures of all the four (**4a**, **4b**, **4c** and **4j**) proposed ligands were drawn using GAUSSVIEW package followed by geometry (Table 4) optimization using GAUSSIAN package (Frisch *et al*., 2009). Hatree-fock theory with "3–21 g" as the basis set was used for optimizing the structures (Fig. 3). Before docking exercise, standard orientation of all the structures after converging to its global energy minima was visualized using PyMOL package (DeLano, 2002) and was saved in PDB format. All

Table 2 The in vitro anti-microbial activity (MIC) of compounds **4a**–**4j**

Compounds	R	R^1	Antibacterial (μgm/mL) MIC				Antifungal (μgm/mL) MIC	
			Gram positive		Gram negative			
			S. aureus	*B. subtilis*	*E. coli*	*Pseudomonas*	*C. albicans*	*A. niger*
4a	5-Me	H	0.2	0.4	0.2	100	0.2	0.2
4b	6-Me	H	0.2	0.2	50	50	0.2	0.2
4c	5-OMe	H	0.4	1.6	50	50	0.2	0.2
4d	4,5-Benzo	H	0.4	3.12	100	100	0.4	0.2
4e	6,7-Benzo	H	3.12	6.25	6.25	–	0.2	0.2
4f	5-Me	Br	1.6	12.5	50	50	0.2	0.2
4g	6-Me	Br	3.12	0.2	12.5	–	0.2	0.2
4h	5-OMe	Br	1.6	100	–	–	0.2	0.8
4i	4,5-Benzo	Br	0.2	6.25	100	–	0.2	0.2
4j	6,7-Benzo	Br	0.2	0.8	50	100	0.4	0.2
Ciprofloxacin			2	2	2	<4	–	–
Fluconazole			–	–	–	–	16	8

Table 3 In vitro anticancer activities against *HeLa* cell lines with compounds **4a–4j**

Compounds	IC_{50} (μg/ml)
4a	20
4b	25
4c	50
4d	50
4e	>50
4f	>50
4g	>50
4h	>50
4i	>50
4j	25

the structurally optimized ligands were later docked using HEX software (v8.0) (Ritchie and Venkatraman, 2010) onto the crystal structure of C-terminal domain (Fig. 4) of gyrase type IIA topoisomerase from *Mycobacterium tuberculosis* (PDB ID: 4G3N) (Bouige *et al*., 2013). Dock energies were calculated based on shape and electrostatics using default grid spacing of 6.0 Å. Among the four ligands that were docked, the one which had the lowest dock energy was selected for further analysis.

Among the four ligands that were docked (Table 5) onto the crystal structure of C-terminal domain of gyrase type IIA topoisomerase from *Mycobacterium tuberculosis*, ligand four got docked with the lowest dock energy (-333.8 kcal mol^{-1}). Upon closer examination through PyMOL package (Fig. 5), residues $arg^{110,186,192}$, $asn^{111,138,188}$, met^{185}, phe^{187}, ile^{189}, asp^{191} of 4G3N were found to interact with ligand **4j** within a distance of 5 Å.

The minimum energy required for the formation of complex between ligand and the receptor (enzyme) indicates excellent binding affinity. However, a low energy indicates that the ligand is fit into the cavity site of the receptor.

Therefore, docking scores of **4j** reveal that compound **4j** is well accommodated in active site of enzyme, and the binding pattern of compounds **4j** showed that it is strongly interact within the active site of topoisomerase II DNA gyrase enzymes.

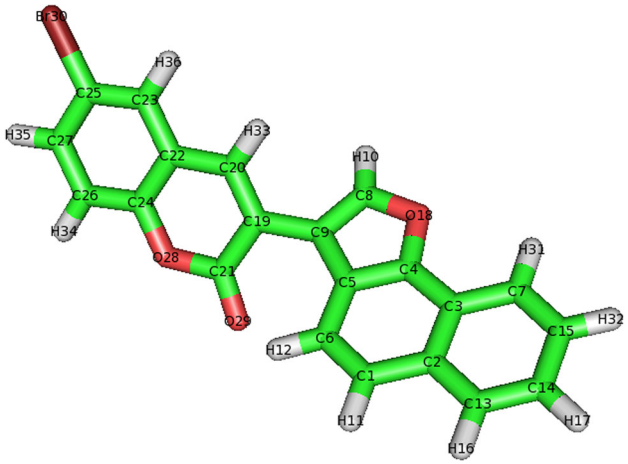


Fig. 3 Geometrically optimized ligand **4j** [E(RHF) = −3580.58 AU after eight cycles; (−2,246,849 kcal $mol^{-1)}$)]. The structure was optimized using GAUSSIAN package (*Hatreefock* theory with "3–21 g" as the basis set). Data in the Table 3 summarize the parameters (*max. force*, *RMS force*, *max displacement and RMS displacement*) considered by GAUSSIAN for the convergence, which is lesser than the threshold value. The image was generated using PyMOL package

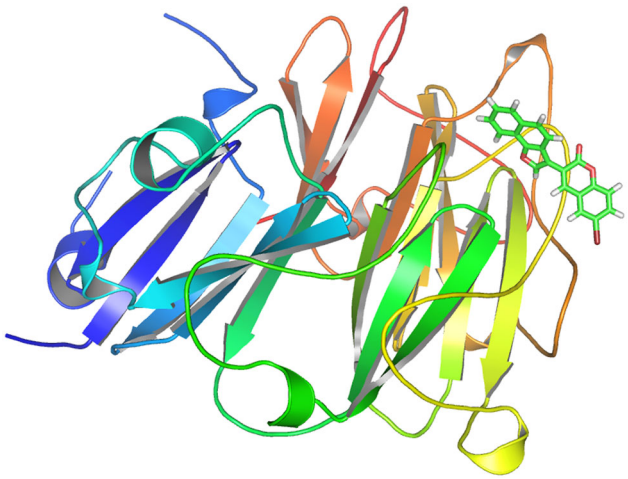


Fig. 4 Docking of geometrically optimized ligand **4j** (*green coloured*) onto the crystal structure of C-terminal domain of gyrase type IIA topoisomerase from *Mycobacterium tuberculosis*. (Dock energy = −338.8 kcal mol^{-1}). The image was generated using PyMOL package

Table 4 The geometrical optimization of the selected Ligand **4j**

Item	Value	Threshold	Converged
Maximum force	0.000036	0.000450	Yes
RMS force	0.000007	0.000300	Yes
Maximum displacement	0.000999	0.001800	Yes
RMS displacement	0.000246	0.001200	Yes

Table 5 Summary of the dock energies computed by the HEX software (v 8) while docking the ligands (A1 to A4) on the crystal structure of C-terminal domain of gyrase type IIA topoisomerase from *Mycobacterium tuberculosis*

Ligand	Dock energy (kcal mol^{-1})
4a	−249.2
4b	−247.2
4c	−250.9
4j	−333.8

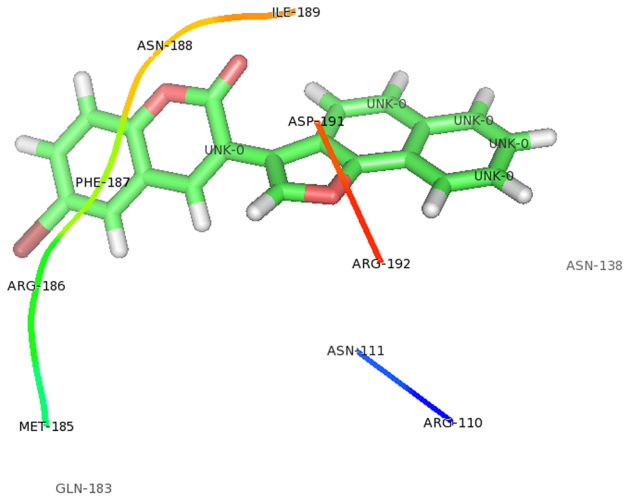


Fig. 5 Residues of crystal structure of C-terminal domain of gyrase type IIA topoisomerase from *Mycobacterium tuberculosis* interacting (distance = 5 Å) with the ligand **4j**. The image was generated using PyMOL package

Conclusions

In summary, we have synthesized series of compounds with simple experimental methods and also optimized the condition with comparing other methods and characterized all the isolated compounds by spectral analysis and some of them by single-crystal X-ray study. The synthesized compounds were carried out anti-microbiological activity, anticancer activity and molecular docking study. The results are extremely good; hence, the present investigation shows the enhancement of anti-microbiological activity by C3 substitution of benzofuran heterocycles.

Supplementary material

Supplementary data associated with this article and crystal data can be attached with manuscript.

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