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Research Article

Synthesis, *in vitro* Antibacterial and Cytotoxic studies of novel Naphthofurans

K. Mathiyazhagan[*], P. Arjun[$] and S. Vennila[#]

[*]*Sri Ganesh Labs Pvt Ltd, Chemicals Research Division, Auto Nagar Industrial Park, Old Gajuwaka, Visakhapatnam-530012, India.*
[$]*Natural Products and Biotechnology Laboratory, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-600 025, India.*
[#]*King Institute of Preventive Medicine, Guindy, Chennai – 600033, India*

Abstract

A simple, convenient and reproducible naphthofuran derivatives were synthesized from 1,4-naphthoquinones and characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, and Mass spectral studies. All the newly synthesized compounds were evaluated for *in vitro* antibacterial activity against Gram positive bacteria and Gram negative bacteria and their minimal inhibitory concentrations were determined. All the compounds were evaluated for in vitro cytotoxicity potential using the standard MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against human cervical cancer cell line (HeLa).

Keywords: Naphthofurans; Spectral studies; Antibacterial; Cytotoxicity

1. Introduction

Furan is an important five-membered oxygen heterocycle in natural and non-natural organic compounds. Among the furan derivatives, naphthofurans have attracted the attention of basic drug researchers owing to their well pronounced activities such as antifungal, cytotoxic [1] and anticancer [2]. Antibacterial activity of 5-hydroxy-1,4-naphthoquinone (Juglone) showed strong inhibition against gram positive intestinal bacteria, *Clostridium perfringens* and weak inhibition against another gram positive intestinal bacteria *Lactobacillus casei* [3]. Comparison of bacterial inhibition of 5-hydroxy-1,4-naphthoquinone with its derivatives revealed that higher inhibition was observed for derivatives having hydroxyl, methyl, and methoxy groups [4], which indicated that the presence of hydroxyl, methyl, and methoxy groups in naphthoquinone are responsible for increasing antibacterial activities. Based on the above results, synthesis and antibacterial screening of various substituted naphthofurans were reported by many research groups [5-14]. But none of them showed significant antibacterial activity. Hence, the discovery of potent furan derivatives from naphthoquinones will be a great advancement in bacterial infection therapies.

In addition to the bacterial diseases, cancer is also a common threat to mankind in present days. Among the cancers, carcinoma of the uterine cervix is the second most common cancer in women, and is the most prevalent female malignancy in many developing countries [15]. It is generally accepted that the early stage cervical cancer can be cured by radical surgery or radiotherapy, while chemotherapy or neoadjuvant chemotherapy are always the first choice for those patients with advanced cervical cancer, where the prognosis remains very poor [16-17]. Although the efficacy of chemotherapy for the majority of cancer types has improved over the last three decades, high toxic effects of chemotherapeutic drugs cause a severe reduction in quality of life which is still formidable problem in clinical medicine [18].

So far majority of 1,4-naphthoquinone derivatives have been synthesized in such a way without affecting the ketone functionality of naphthoquinones on either at 1st or 4th positions or on both. No information was available about the biological activities of the naphthofurans, where the ketone functionality is totally absent 1st or 4th position.

* Correspondence Info
Dr. K. Mathiyazhagan
Sri Ganesh Labs Pvt Ltd,
Chemicals Research Division, Auto Nagar Industrial Park,
Old Gajuwaka, Visakhapatnam-530012, India
E-mail: mathi10789@yahoo.co.in

Moreover, among the various regioisomeric forms of naphthofuran (Fig. 1), the derivatives of almost all the isomeric forms have been synthesized either by synthetic or semi-synthetic methods and screened for biological activities, but the compounds reported on naphtho[1,2-*b*]furan derivatives are less. Therefore, we decided to synthesize various naphtho[1,2-*b*]furans and study of their *in vitro* antibacterial and cytotoxic activities.

2. Material and Methods

2.1 Experimental

Chemicals were purchased from Alfa-Aesar or Sigma-Aldrich and were used without further purification. The melting points were determined with a Buchi-540 by open capillary method and are uncorrected. IR spectra were recorded on a PARAGON 100 FT-IR spectrophotometer using KBr pallet technique. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solvent on a JEOL 300 MHz and BRUKER 500 MHz instruments respectively. The chemical shifts are reported in δ (ppm) using and tetramethylsilane (TMS) as an internal standard. The splitting patterns were designated as follows; s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet. Mass spectrum was obtained using a GCMS-QP 2010 (Shimadzu, Japan) equipped with DI source and TIC detector.

2.1.1 Synthesis of Diethyl-7-hydroxynaphtho[1,2-b:4,3-b']difuran-3,4-dicarboxylate (1)

5-Hydroxy-1,4-naphthoquinone **6** (1.40 g, 8.04 mmol) was added into a solution of Ethyl-N,N-dimethylamino acrylate **7** (1.38 g, 9.64 mmol) in Acetic acid (30 ml) and stirred continuously for 12 hours at room temperature. The reaction mixture was diluted with Water and extracted with Ethyl acetate (3x25mL). The combined organic layer was washed with Water, dried over anhydrous Sodium sulphate and the solvent was evaporated. The residue was purified by column chromatography on silica gel using n-Hexane and Ethyl acetate as eluents to afford **1** (2.05 g, 70%). **Mp** 122-125 °C; **IR** (KBr, cm^{-1}): 3079 (aromatic C-H stretching), 1741, 1726 (C=O stretching), 1643, 1457 (aromatic C=C stretching), 1253, 1186 (C-O stretching); **^{1}H-NMR** ($CDCl_3$, δ ppm): 1.43 (6H, t, *J* = 9Hz, 2xCH_3), 4.52 (4H, q, *J* = 9Hz, 2x CH_2), 7.33 (1H, d, *J* = 3Hz, 1xAr-H), 7.70 (1H, t, *J* = 6Hz, 1xAr-H), 7.85 (1H, d, *J* = 4.5 Hz, 1xAr-H), 8.31 (1H, s, 1xAr-H), 8.95 (1H, q, *J* = 3Hz, 1xAr-H), 12.09 (1H, s, OH, exchangeable); **^{13}C-NMR** ($CDCl_3$, δ ppm): 14.0, 14.3, 62.3, 62.5, 115.7, 120.1, 124.9, 129.2, 133.1, 133.5, 133.9, 134.2, 135.4, 135.7, 137.3, 162.7, 164.0, 168.5, 181.0, 186.9; **DI-MS (70eV), m/z**: 368 (M^+, 50%), 323 (M^+ -[OC_2H_5], 100%), 295 (M^+ -[$COOC_2H_5$], 48%), 266 (M^+ -[$COOC_2H_5$] -[C_2H_5], 15%), 251 (M^+ -[$COOC_2H_5$]-[OC_2H_5]+1, 13%), 221 (M^+ -[$COOC_2H_5$]$_2$ -1, 10%).

2.1.2 Synthesis of 4-Ethoxycarbonyl-7-hydroxynaphtho[1,2-b:4,3-b']difuran-3-carboxylic acid (2)

An aqueous solution of Sodium hydroxide (0.456 g, 11.4 mmol in 2.0 mL of Water) was added into a solution of **1** (1.40 g, 3.8 mmol) in Ethanol (15.0 mL) and the reaction mixture was heated to reflux (~85 °C) and maintained for 5 hours. After the completion of the reaction (by TLC), Water (50 mL) was added and the reaction mixture was extracted with Ethyl acetate (3x10 mL). The combined organic layer was dried over anhydrous Sodium sulphate and the solvent was evaporated. The residue was purified by column chromatography on silica gel using n-Hexane and Ethyl acetate as eluents to afford **2** (620 mg). **Mp** 225-230 °C; **IR** (KBr, cm^{-1}): 3084 (aromatic C-H stretching), 1736, 1701 (C=O stretching), 1644, 1459 (aromatic C=C stretching), 1272, 1193 (C-O stretching); **^{1}H-NMR** (DMSO-d_6, δ ppm): 1.33 (3H, t, *J* = 3Hz, 1xCH_3), 4.42 (2H, q, *J* = 6Hz, 1x CH_2), 7.43 (1H, d, *J* = 9Hz, 1xAr-H), 7.71-7.85 (2H, m, 2xAr-H), 8.26 (1H, s, 1xAr-H), 8.74 (1H, s, 1xAr-H), 12.14 (1H, s, OH, exchangeable), 14.0 (1H, bs, OH, exchangeable); **^{13}C-NMR** (DMSO-d_6, δ ppm): 14.3, 62.2, 116.7, 119.8, 124.9, 128.7, 132.7, 133.0, 133.4, 134.6, 135.2, 136.5, 137.9, 161.8, 165.6, 168.2, 181.2, 186.7; **DI-MS (70eV), m/z**: 340 (M^+, 42%), 295 (M^+ -[OC_2H_5], 100%), 266 (M^+ -[$COOC_2H_5$]-1, 38%).

2.1.3 Synthesis of 7-Hydroxynaphtho[1,2-b:4,3-b']difuran-3,4-dicarboxylic acid (3)

The aqueous layer obtained after the isolation of compound **2** was acidified using conc. HCl and the solid formed was filtered (0.410 g). The pale brown solid was purified by column chromatography on silica gel using Chloroform and Ethyl acetate as eluents to afford **3** (340 mg). **Mp** above 300 °C (with dec); **IR** (KBr, cm^{-1}): 3074 (aromatic C-H stretching), 1698, 1639 (C=O stretching), 1596, 1454 (aromatic C=C stretching), 1276, 1233 (C-O stretching); **^{1}H-NMR** (DMSO-d_6, δ ppm): 7.42 (1H, d, *J* = 6Hz, 1xAr-H), 7.69 (1H, d, *J* = 6Hz, 1xAr-H), 7.84 (1H, d, *J* = 9Hz, 1xAr-H), 8.19 (1H, s, 1xAr-H), 8.71 (1H, s, 1xAr-H), 12.15 (1H, s, OH, exchangeable) and 13.74 (2H, bs, OH, exchangeable); **^{13}C-NMR** (DMSO-d_6, δ ppm): 116.3, 119.8, 124.7, 128.0, 132.6, 132.9, 133.7, 134.3, 136.2, 136.9, 137.9, 161.8, 165.6, 169.7, 181.4, 187.1; **DI-MS (70eV), m/z**: 312 (M^+, 62%) 294 (M^+ -[OH], 28%), 268 (M^+ -[COOH]+1, 100%), 251 (M^+ -[COOH]-[OH]+1, 15%), 223 (M^+ -[COOH]$_2$ +1, 30%).

2.1.4 Synthesis of Diethylnaphtho[1,2-b:4,3-b']difuran-3,4-dicarboxylate (4) and Ethyl-5-hydroxy naphtho[1,2-b]furan-3-carboxylate (5)

1,4-Naphthoquinone **8** (2.0 g; 12.6 mmol) was added into a stirred solution of Ethyl-N,N-dimethylamino acrylate **7** (2.17 g, 15.2 mmol) in Acetic acid (40 mL) and stirred continuously for 6 hours at room temperature. The reaction mixture was diluted with water and extracted with Ethyl acetate (3x25mL). The combined organic layer was dried over anhydrous Sodium sulphate and the solvent was evaporated to get 2.80 g of residue, which showed two equal intensity spots by TLC. The residue was purified by column chromatography on silica gel using n-Hexane and Ethyl acetate as eluents to afford compounds **4** (440 mg) and **5** (520 mg).

Analytical data for 4: **Mp** 155-158 °C; **IR** (KBr, $cm^{-1)}$: 3073 (aromatic C-H stretching), 1724 (C=O stretching), 1589, 1454 (aromatic C=C stretching), 1265 (C-O stretching); **^{1}H-NMR** (DMSO-$d_{6,}$ δ ppm): 1.32-1.40 (6H, m, 2xCH_3), 4.38-4.45 (4H, m, 2xCH_2), 7.96 (2H, d, *J* = 4.2Hz, 2xAr-H), 8.17-8.27 (3H, m, 3xAr-H), 8.71 (1H, s, 1xAr-H); **^{13}C-NMR** (DMSO-$d_{6,}$ δ ppm): 14.3, 14.5, 62.2, 62.5, 127.4, 127.5, 128.5, 132.4, 133.2, 133.4, 134.7, 134.9, 135.3, 135.4, 135.5, 164.2, 168.2, 181.6, 182.0; **DI-MS (70eV), m/z**: 352 (M^+, 10%), 307 (M^+ -[OC_2H_5], 85%), 279 (M^+ -[$COOC_2H_5$], 45%), 252 (M^+ -[$COOC_2H_5$]-[C_2H_5]+2, 20%), 235 (M^+ -[$COOC_2H_5$]-[OC_2H_5]+1, 22%), 207 (M^+ -[$COOC_2H_5$]$_2$ +1, 12%).

Analytical data for 5: **Mp** 182-185 °C; **IR** (KBr, $cm^{-1)}$: 3057 (aromatic C-H stretching), 1690 (C=O stretching), 1591, 1451 (aromatic C=C stretching), 1249 (C-O stretching); **^{1}H-NMR** (DMSO-$d_{6,}$ δ ppm): 1.36 (3H, t, *J* = 6.9Hz, 1xCH_3), 4.35 (2H, q, *J* = 3.6Hz, 1xCH_2), 7.43 (1H, s, 1xAr-H), 7.55 (1H, t, *J* = 7.5Hz, 1xAr-H), 7.65 (1H, t, *J* = 7.5Hz, 1xAr-H), 8.16 (1H, d, *J* = 7.5Hz, 1xAr-H), 8.24 (1H, d, *J* = 9Hz, 1xAr-H), 8.73 (1H, s, 1xAr-H), 10.42 (1H,bs, OH, exchangeable); **^{13}C-NMR** (DMSO-$d_{6,}$ δ ppm): 14.7, 60.7, 100.0, 115.1, 119.7, 121.0, 121.4, 123.7, 123.8, 125.4, 127.8, 145.1,151.2, 151.3, 163.4; **DI-MS (70eV), m/z**: 256 (M^+, 100%), 228 (M^+ -[C_2H_5]+1, 80%), 211 (M^+ -[OC_2H_5], 25%), 183 (M^+ -[$COOC_2H_5$], 10%).

2.2 Antibacterial studies

All the synthesized naphthofuran derivatives were tested for *in vitro* antibacterial activity against the following microorganisms: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 155) and *Bacillus cereus* (ATCC 11778) (Gram-positive bacteria), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 29665) and *Pseudomonas aeruginosa* (ATCC 25619) (Gram-negative bacteria) by cup plate method [20-21]. The minimal inhibitory concentration (MIC) values for compounds tested, defined as the lowest concentration of the compound preventing the visible growth, were determined by using serial dilution method [20].

2.2.1 Sample preparation and In vitro antibacterial activity

The sterile nutrient agar plates were prepared by pouring the sterile into petri dishes in aseptic conditions. Overnight cultures of bacteria organisms were adjusted to 106 cfu ml^{-1} according to the Mc Farland turbidity standards. Each standardized test organism culture (100 µL) was spread onto the agar plates. All the compounds were dissolved in DMSO of 5 mg/mL. Empty sterilized discs of 6 mm (Himedia, Mumbai, India) were each impregnated with 50 µL of compounds. Discs were placed on agar plates and the cultures were incubated at 37 ºC for 24 h, after which the diameters of the inhibition zones were measured. Each experiment was repeated thrice to minimize the error. Tetracycline was used as a reference standard and DMSO was used as solvent control. The solvent control (DMSO) did not show any antibacterial activity. The minimum inhibitory concentration (MIC) of all the compounds was determined by two-fold serial dilution technique. The study involved a series of six assay tubes for each test compound against six bacterial strains. The entire test was done in duplicate. To the first assay tube, 1.8 mL of seeded broth and 0.2 mL of test compound (1 mg/mL) were added and mixed thoroughly, and the two fold serial dilution was done upto the sixth tube containing 1 mL of the seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. The solvent control, negative control (growth control), and drug control were maintained. The assay tubes were incubated for 24 h at 37 ºC. The lowest concentration which apparently caused complete inhibition of growth of microorganisms was considered as MIC.

2.2.3 *In vitro* cytotoxic activity

The *in vitro* cytotoxic activity of the compounds was carried out against human cancer cell line (HeLa), using MTT assay method. The cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India and was cultured in DMEM medium supplemented with 10% FBS, 1% L-Glutamine and 1% Penicillin G, Streptomycin 100 mg/mL, Amphotericin B antibiotic solution in a CO_2 incubator in a humidified atmosphere of 5% CO_2 and 95% air. The *in vitro* cytotoxicity was determined using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyl tetrazolium bromide (MTT) assay method [22-23]. Briefly, the exponentially growing cells were placed in 96-well plates (10^4 cells/well in 100 µL of medium) and incubated for 24 h. The test compounds were prepared by prior to the study by dissolving in 0.1% of DMSO and diluted with medium. The cells were then exposed to different concentration of test compounds (10, 20 and 50 µM) in a volume of 100 µL/well. The cells in the growth control wells received only the same volume of medium containing 0.1% DMSO. After 72 h of exposure, the medium was removed and the cell cultures were incubated with 100 µl of MTT reagent (0.1%) for 4 hour at 37 °C. The pink colored formazan was dissolved in 100 µL of DMSO and absorbance of each well was read in an ELISA microplate reader at 570 nm. The experiment was performed in triplicate and the percentage cytotoxicity was calculated using the following formula

% Cytotoxicity = (Control absorbance – Test absorbance)/Control absorbance X 100

The drug concentration that causes 50% cell growth inhibition after 72 h of continuous exposure to the test compounds (IC_{50}) was determined by plotting the graph of concentration of the drug against the percent cytotoxicity and performing the regression analysis. The IC_{50} values of the test compounds are shown in Table-2.

3. Results and Discussion

3.1 Synthesis

In order to achieve the scope of this work, 5-Hydroxy-1,4-naphthoquinone (**6**) was reacted with Ethyl-N,N-dimethylamino acrylate (**7**), which afforded novel Diethyl-7-hydroxynaphtho[1,2-*b*:4,3-*b′*]difuran-3,4-dicarboxylate (**1**). Varying molar equivalents of **7** also did not yield monofuran derivative. Hydrolysis of **1** leads to the formation of two novel derivatives viz., 4-Ethoxycarbonyl-7-hydroxynaphtho[1,2-*b*:4,3-*b′*]difuran-3-carboxylic acid (**2**) and 7-Hydroxynaphtho[1,2-*b*:4,3-*b′*]difuran-3,4-dicarboxylic acid (**3**) due to partial and complete hydrolysis of both the ester groups (**Scheme 1**).

Naphtho[1,2-*b*]furan derivative was also prepared by reacting 1,4-Naphthoquinone (**8**) with Ethyl-N,N-dimethylamino acrylate (**7**) to study the antibacterial and cytotoxic activities. In addition to the already reported [19] Ethyl-5-hydroxynaphtho[1,2-*b*]furan-3-carboxylate (**5**), one novel Diethyl naphtho[1,2-*b*:4,3-*b′*]difuran-3,4-dicarboxylate (**4**) was also formed (**Scheme 2**) during this reaction. TLC analysis of crude product showed two equal intensity spots, which were separated by column chromatography. It was also observed that the formation of **4** is inevitable even with low molar equivalents of **7**.

All the compounds **1**-**5** were purified by column chromatography and characterized by ^{1}H NMR, ^{13}C NMR, FT-IR and Mass spectral studies.

The IR spectra of **1**, **2**, **3** and **5** exhibited broad hydroxyl absorption bands around 3650 cm^{-1}, which is absent in compound **4.** This indicated the absence of hydroxyl group in **4**. The bands appeared in compounds **1**, **2**, **4**, and **5** within the region 1724-1741 cm^{-1} are due to the carbonyl stretching frequency of ester groups. Similarly, the compounds containing acid groups **2** and **3** showed lower frequency bands in the region of 1639-1701 cm^{-1}, which are assigned to carbonyl stretching frequencies of acid group. In the case of **3**, the presence of band corresponding to carbonyl group of acid and the absence of bands in the region 1720-1740 cm^{-1} confirm that the ester groups of **1** are completely hydrolysed to carboxylic acid group. The bands in the range of 1186-1276 cm^{-1} are assigned to C-O stretching frequency.

The ^{1}H NMR spectra of the compounds **1**, **2**, **3**, and **5** showed a characteristic D_2O exchangeable proton signal within 10.42-12.15 ppm region due to OH proton. The OH group of carboxylic acid in **2** and **3** appeared as broad singlet at 14.00 and 13.74 ppm, respectively. The methyl and methylene protons of ethyl ester group present in **1**, **2**, **4**, and **5** resonate at 1.32-1.46 and 4.33-4.55 ppm, respectively. The significant change in ^{1}H NMR signals in **3** compared to **1** is due to hydrolysis of ester group in **1** to carboxylic acid group. This was confirmed by the disappearance of methyl and methylene group signals and the appearance of a singlet in the 13.74 ppm for the COOH proton. The aromatic proton signals appeared in the region 7.31-8.95 ppm for all the compounds.

^{13}C NMR of **1**, **2**, **4**, and **5** showed peaks at 14.0-14.7 and 60.7-62.5 ppm for methyl and methylene carbon signals, respectively for ethyl ester groups. These signals are absent in compound **3** which indicates the absence of ester group. In the case of compounds **1**-**4,** two signals observed in the region 161.8-169.7 and 181.0-187.1 ppm are due to the two carbonyl groups. A signal observed in the downfield region at 163.4 ppm is due to the presence of only one carbonyl group in **5.**

Mass spectra of compounds **1-5** exhibited molecular ion peak m/z at 368, 340, 312, 352, and 256 respectively.

3.2 Antibacterial studies

The naphthofuran derivatives (**1-5**) showed good to moderate antibacterial activity at 75 µg ml^{-1} concentration with zone of inhibition in the range of 12-27 mm, the standard drug tetracycline had the zone of inhibition in the range of 18-25 mm. Among the tested compounds, **1** showed good antibacterial activity with zone of inhibition in the range of 12-27 mm at 75 µg ml^{-1} concentration against the tested organisms. The compounds **2** and **5** showed moderate antibacterial activity with zone of inhibition in the range of 16-26 mm at 75 µg ml^{-1} concentration against the tested organisms. Rest of the compounds **3** and **4** showed comparatively weak antibacterial activity with zone of inhibition in the range of 14-21 mm. The minimum inhibitory concentrations (MICs) of all the compounds **1-5** were also studied by two-fold serial dilution method [20] and the MIC data are recorded in Table-1. The compound **1** exhibited lowest MIC and the compound **4** showed lesser activity against all the tested organisms. The rest of the compounds **2**, **3**, and **5** exhibited moderate activity. The investigation of structure activity relationship clearly revealed that the higher antibacterial activity of compounds **1**, **2**, **3** and **5** may be attributed due to the presence of hydroxyl group. As the compound **4** is not having hydroxyl group, showed comparatively lesser activity. This study indicated that hydroxyl substitution at aromatic ring enhances the antibacterial activity against the tested organisms. Suitable structural modification of these compounds may lead to potent antibacterial agents in future.

3.3 Cytotoxicity

The cytotoxicity of all the compounds was evaluated *in vitro* against HeLa cancer cell line. The standard MTT assay was used to determine IC_{50} values, i.e., the drug conc. that causes 50% cell growth inhibition after 72 h of continuing exposure to the test compounds and the mean of the results obtained from triplicate assays are shown in Fig. 2.

Microscopic images of control cancer cells and apoptotic morphological changes in HeLa cell line treated with compounds **1-5** are shown in Fig. **3**. All the compounds revealed a dose dependant inhibition of growth of the cells. The IC_{50} values of all the compounds demonstrated significant cytotoxic activity except **5**. Among these compounds, **3** showed the more potent activity, where one hydroxyl and maximum number of two carboxylic acid groups are present. The IC_{50} of these compounds were comparable with known anticancer agent, Cisplatin (IC_{50} 13.54 µg/mL). The present study revealed that the tested cancer cell line, HeLa is slightly more sensitive to all the tested compounds. The results indicated that the cytotoxicity depends on the nature of the substituent present in the derivatives.

As evident from the results represented in Fig. **3**, the tested naphthofuran derivatives (**1-5**) demonstrated cytotoxic effects against HeLa cell line. 7-Hydroxynaphtho[1,2-*b*:4,3-*b'*] difuran-3,4-dicarboxylic acid (**3**) was proved to be the most active cytotoxic agent amongst the synthesized naphthofuran derivatives, causing 50% inhibition of cell viability at low concentration (56.74 µg/mL). Ethyl-5-hydroxynaphtho[1,2-*b*]furan-3-carboxylate (**5**) was proved to be the least active. The cytotoxity of the tested compounds decreases in the following order: **3 > 1 > 2 > 4** > **5**.

In conclusion, we report herein a simple, convenient and reproducible method for the synthesis of several naphthofuran derivatives of which the compounds having hydroxyl substitution at aromatic ring showed better activity. Cytotoxicity depends on the nature of the substituents present in the derivatives. These observations are useful to design and synthesize further new lead antibacterial and cytotoxic compounds.

Table 1: *In vitro* anti-bacterial activity of synthesized Naphthofuran derivatives (1-5)

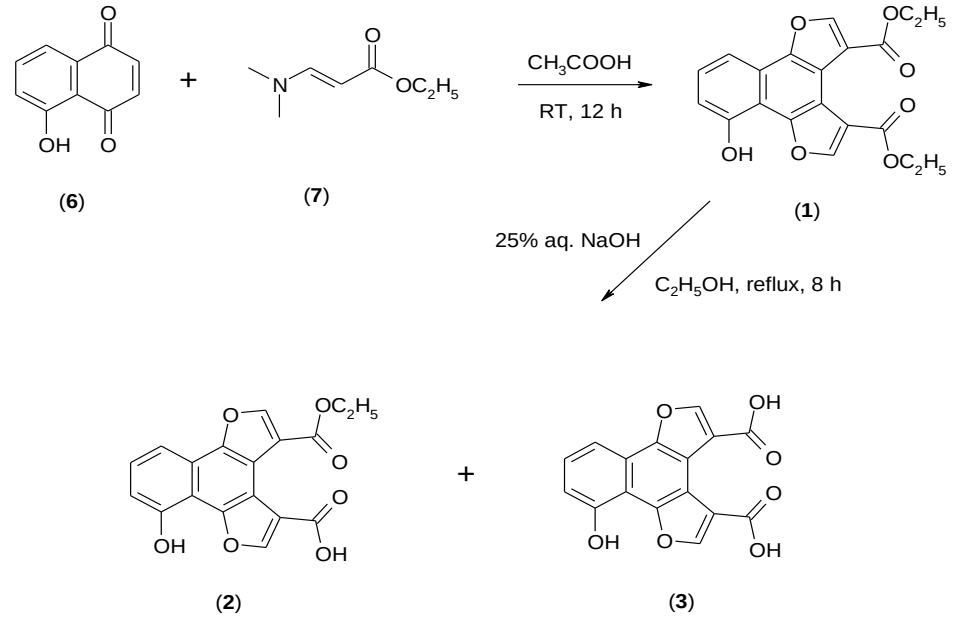
Compound	Minimum inhibitory concentration (in µg mL^{-1})					
	S. aureus	***S. epidermidis***	***B. cereus***	***E. coli***	***K. pneumonia***	***P. aeruginosa***
1	9	7	9	8.5	10.5	7
2	18	12	16	20	12	10.5
3	14	15	14	15	14	9
4	20	18	16	22	20	17.5
5	16.5	14	15.5	13	11	13.5
Tetracycline	0.015	0.016	0.014	-	0.016	0.014

Table 2: *In vitro* cytotoxicity activity of Naphthofuran derivatives (1-5) in HeLa

Compound	IC_{50} (µM)[a]
1	72.43 ± 1.53
2	82.14 ± 1.48
3	49.48 ± 1.65
4	92.76 ± 1.92
5	132.41 ± 2.43
Cisplatin	13.54 ± 0.01

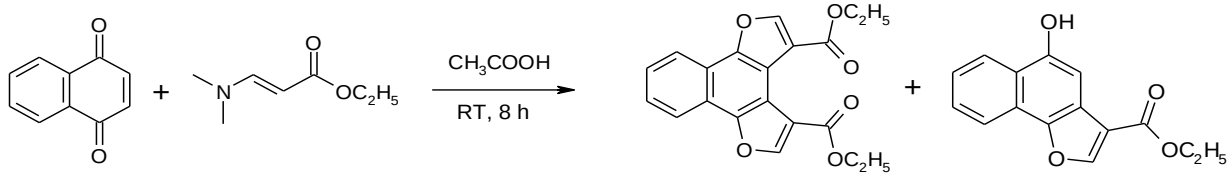
[a] The drug concentration that causes 50% cell growth inhibition.

Scheme 1: Synthesis of Naphthofuran derivatives (Preparation of compounds 1-3)



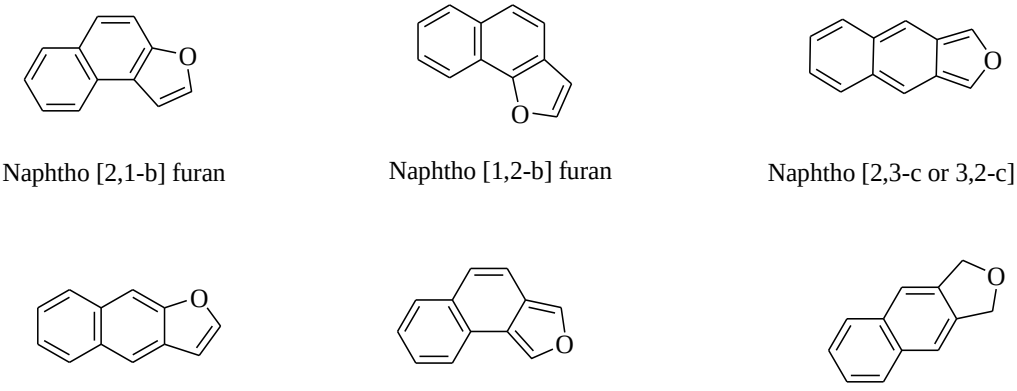
(**6**) (**7**) (**1**) (**2**) (**3**)

Scheme 2: Synthesis of Naphthofuran derivatives (Preparation of compounds 4 and 5)



(**8**) (**7**) (**4**) (**5**)

Figure 1: Various regioisomeric forms of Naphthofurans



Naphtho [2,1-b] furan | Naphtho [1,2-b] furan | Naphtho [2,3-c or 3,2-c] furan

Naphtho [2,3-b or 3,2-b] furan | Naphtho [1,2-c or 2,1-c] furan | 1,3-Dihydronaphtho[2,3-*c*]furan

Figure 2: Cytotoxicity of Naphthofuran derivatives (1-5) against cervical cancer cell line.

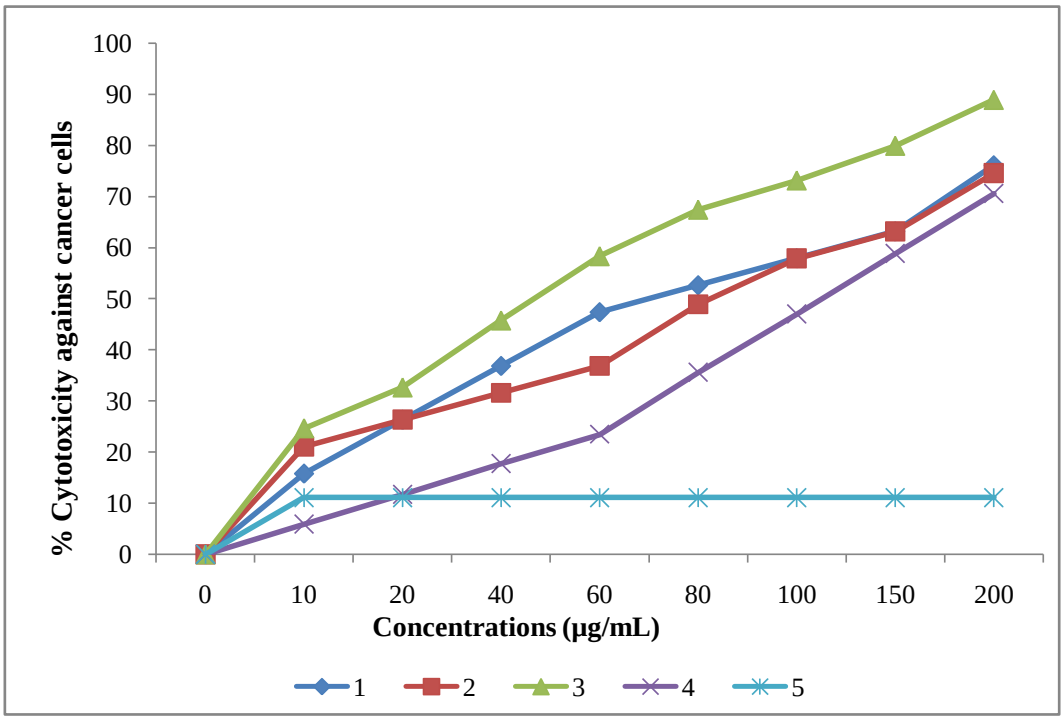
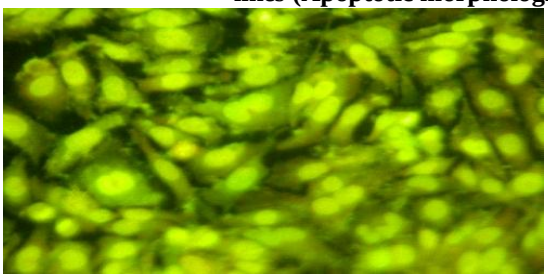
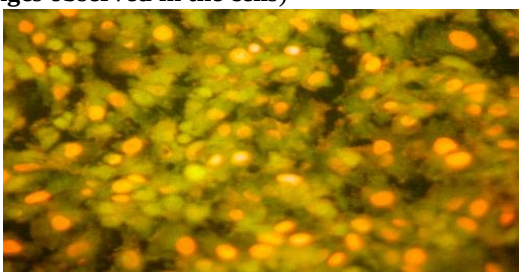


Figure 3: Cell damage/ cell death observed in Naphthofuran derivatives (1-5) against cervical cancer cell lines (Apoptotic morphological changes observed in the cells)



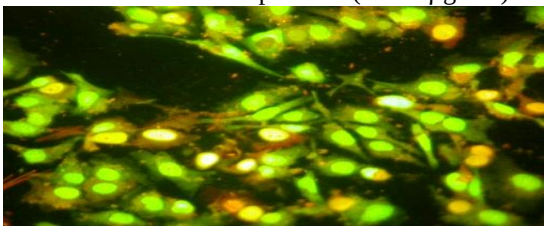
a. Control cancer cell



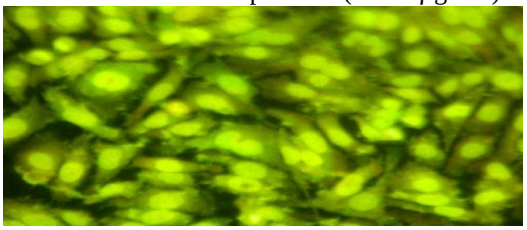
b. Treated with compound 1 (72.43 µg/mL)

c. Treated with compound 2 (82.14 µg/mL)

d. Treated with compound 3 (49.48 µg/mL)



e. Treated with compound 4 (92.76 µg/mL)



f. Treated with compound 5 (132.41 µg/mL)

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