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A simplified synthetic route and study of cytotoxicity of Ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylate and its methyl derivative

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Abstract: Preparation of a biologically active benzofuran derivative ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitro-benzofuran-3-carboxylate from commercially available starting materials 2-bromo-4-methoxy-phenol and *para*-fluorophenylacetylene is described in four steps with good yields. The final compound and all the intermediates reported in the method were characterized by spectroscopic techniques. Finally, the target molecule and its methyl derivative were tested for their anticancer activity and were found to be 95.2 μg/mL and 8.5 μg/mL (IC_{50}) respectively against HCT 116 cell line.

Keywords: Anticancer; HCT116; Ethyl 2-(4-fluorophenyl)-5-hydroxy-6- nitrobenzofuran-3- carboxylate; 2-bromo-4-methoxy-phenol; MTT.

1. Introduction

In organic chemistry, the heterocyclic moieties occupy a central position[1-6], further these ring systems have also emerged as powerful scaffolds for many biologically important molecules[7-10]. Moreover, it is known that in the design and discovery of new active drug molecules the heterocycles played an important key role[11].

2. Background

In heterocyclic chemistry, the benzofuran derivatives constitute one of the major class and a survey of literature revealed that a good number of reports were shown that benzofuran derivatives with valuable biological activities. These derivatives had potent anti-tumor, anti-viral properties, and also shows DNA binding affinities[12,13] The following structures I, II, III and IV (Figure 1) are the important benzofuran analogues[14-16] have both activities. Further, the broad spectrum of pharmacological activities of individual benzofurans stipulates that it is worth to consider those moieties with benzofuran nucleus shows higher biological activity. So, encouraged by the activities of

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the benzofuran analogous in the present investigation we aimed to develop a simple synthetic approach and to study the anticancer activity of an important active benzofuran derivative Ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylate**.** The simplified method involves preparation from commercially available starting material 2-bromo-4-methoxy-phenol **(1)** as shown in Scheme 1. Finally, as per the aim of our study, the anti cancer activity of titled compound **8** was studied along with its methyl derivative **7** obtained in the synthesis against HCT 116 cell line.

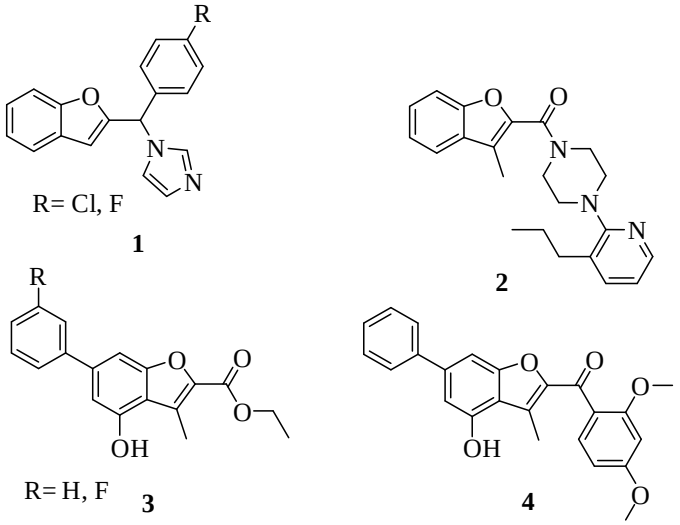


Figure 1. Biologically important benzofuran analogs

3. Experimental

General: The chemicals 2-bromo-4-methoxy-phenol, Triphenyl phosphine are purchased from TCI America, Palladium acetate from Alfa-Aesar, Triphe from TCI America, Boron trichloride from Sigma-Aldrich, (4-Fluorophenyl)-acetylene and ethyl chloroformate from Merck manufactures were obtained and used as such. The ^{1}H NMR, ^{13}C NMR are recorded on BRUKER 400 MHz spectrometer at 400/300 MHz and 100 MHz frequencies respectively.

Synthetic procedure: The total synthesis of compound **8** is described (Scheme 1) in four steps excluding the step involving preparation of compound **5** from compound **4.**

Preparation of 2-bromo-4-methoxy-5-nitrophenyl acetate (2): To a solution of 2-bromophenol (1 g, 1 eq.) and added mixture of solvents acetic anhydride and acetic acid (1:1) (1.5 mL) and refluxed at 110 °C for 16 hours and cooled to -10 °C and added fuming nitric acid (0.65 mL, 1.5 eq.) slowly and stirred for 2 hours and poured into ice cold water to obtain compound **2** as a yellow colored precipitate (1.31 g, 92 %), which is a novel intermediate compound found to be useful for the preparation of further new moieties.

*2-Bromo-4-methoxy-5-nitrophenyl acetate **(2)*****:** Yellow colored solid. Yield: 1.31 g, 92 %. M.P. 255-260 ^{o}C. ^{1}H NMR: in ($CDCl_3$, 300MHz): δ 7.8 (s, 1H), 7.3 (s, 1H), 4.0 (s, 3H), 2.5 (s, 3H). Mass: *m/z*; 289, 291.

*Preparation of acetate of 2-bromo-4-methoxy-5-nitrophenol **(3)*****:** The compound **2** (1 g, 1 eq.) thus obtained is dissolved in THF, methanol, and water (1:1:2) (40 mL) and lithium hydroxide (480 mg, 6 eq.) was added and stirred at room temperature for 3 hours and the reaction progress was monitored by thin layer chromatography and after completion the reaction mixture was neutralized by 1 M HCl solution. The crude compound is extracted with ethylacetate and is dried over anhydrous sodium sulphate and concentrated to obtain the de-acetylated product **3**. The structure of the compound was confirmed by ^{1}H NMR and mass spectral data.

*Acetate derivative of 2-bromo-4-methoxy-5-nitrophenol **(3)*:** Yield: Pale yellow colored solid. Yield: 730 mg, 85 %. M.P. 305-310 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.6 (s, 1H), 7.2 (s, 1H), 5.5 (bs, 1H, D_2O exchangeable), 4.0 (s, 3H). Mass: *m/z*: 247, 249, 248.

*Preparation of ethyl-(p-fluorophenyl propynoate) (**5**)*: The other starting material 4-fluorophenylacetylene (**4**) (5 mL, 1 eq.) is dissolved in dry diethyl ether (150 mL) and cooled to -78 °C (by using dryice + acetone mixture) and then added n-butyl lithium (5 mL, 1.1 eq.) slowly drop by drop for lithiation and was quenched by the addition of ethylchloroacetate (5 mL, 1.1 eq.) by drop wise and the reaction was bring to room temperature and allowed to stir for 2 hours and progress was monitored by TLC and the reaction mixture was quenched by saturated ammonium chloride and extracted with ethylacetate and dried over anhydrous sodium sulphate and concentrated to get compound **5** in 93%**.** The crude compound was purified by column chromatography. The compound was characterized by ^{1}H NMR and mass spectral data.

*Ethyl-(p-fluorophenyl propynoate) (**5**)*: White colored solid. 750 mg, 93%. M.P. 260-263 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.6 (d, 2H), 7.1 (d, 2H), 4.2 (q, 2H), 1.7 (t, 3H). Mass: *m/z*: 193, 194.

*Ethyl ester of 2-(4-fluorophenyl)-5-methoxy-6-nitrobenzofuran-3-carboxylicacid (**7**)*: To the compound **3** (**6**00 mg, 1 eq.) added compound **5** (553 mg, 1.2 eq.) (obtained from 4-fluorophenylacetylene) and potassium carbonate dissolved in to acetonitrile solvent (6 mL) and stirred for 15 minutes and then the mixture was purging with nitrogen gas for degasification to get the intermediate **6** which would be further undergoes intra molecular Heck reaction by the addition of palladium acetate (27 mg, 0.05 eq.), triphenylphosphine (TPP) (628 mg, 1 eq.) and stirred for 16 hours at 110 °C and the reaction was monitored by TLC. After completion of the reaction, the mixture was worked up with water and extracted with ethyl acetate and dried over sodium sulphate and concentrated. The crude compound was purified by column chromatography to get product **7** and the compound is confirmed by ^{1}H NMR and mass spectral data.

*Ethyl 2-(4-fluorophenyl)-5-methoxy-6-nitrobenzofuran-3-carboxylate (**7**)*: White colored solid. Yield: 660 mg, 75%. M.P. 265-267 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.1 (s, 1H), 8.0 (d, 2H), 7.8 (s, 1H), 7.2 (d, 2H), 4.4 (q, 2H), 4.0 (s, 1H), 1.4 (t, 3H). Mass: *m/z*: 360, 361, 362.

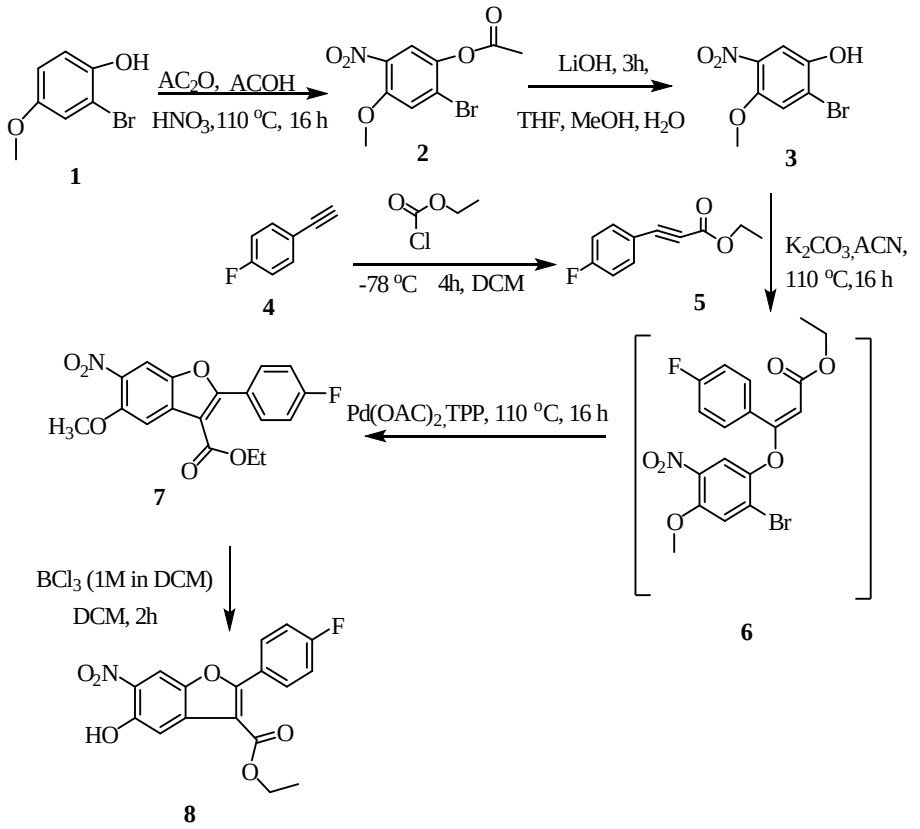
Preparation of Ethyl ester of 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylic acid **(*8*)**: Finally the compound **7** (500 mg, 1eq) in dichloromethane (40 mL) at 0 °C was treated with BCl_3 (0.3 mL, 2.5 eq) slowly by drop wise, and the reaction mixture was stirred for 2 hours at room temperature and the reaction progress was monitored by TLC. Later the reaction mixture was quenched by the addition of sodium bicarbonate and the compound was extracted by using dichloromethane. The dichloromethane layer was dried over anhydrous sodium sulfate and concentrated to get the final product **8** and was confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectral data.

*Ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylate **(8)***: Yellow colored solid. Yield: 320 mg, 65 %. M.P. 365-368 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11 (bs, 1H, D_2O exchangeable), 8.3 (s, 1H), 8.0 (d, 2H), 7.6 (s, 1H), 7.4 (d, 2H), 4.3 (q, 2H), 1.3 (t, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 162, 161, 160, 149, 147.1, 131.7, 130.7, 128.6, 117.9, 116.0, 109.7, 108.3, 59.1, 13.6. Anal. calc. for $C_{17}H_{12}FNO_6$: C, 59.14; H, 3.50; F, 5.50; N, 4.06. Found: C, 59.07; H, 3.59; F, 5.44; N, 4.11. Mass: *m/z*- 346.29 (100 %), 347.31, 348.07, 344.34.

Methodology for Cytotoxic activity:** The compounds were tested on HCT116 cells using MTT cell proliferation assay[17], HCT116 cell line was obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's medium (DMEM) (Sigma Life Science, USA) containing 10 % fetal bovine serum (FBS). The cells (2,000 cells per well) were seeded in a 96-well micro plate containing 100 μL of DMEM complete medium per well and incubated at 37 °C with 5 % CO_2. The cells were treated different concentrations of compounds up to 72 hours for every 24 hours interval. Controls were maintained with 0.5 % DMSO. After 72 hours treatment, 5 µL of MTT

(3-(4,5-dimethyl- thiazol-2-yl)- 2,5-diphenyl tetrazolium bromide) reagent (R&D Systems, USA) along with 45 µL of phenol red free DMEM (Sigma Life Science, USA) without FBS was added to each well and plates were incubated at 37 ^{o}C with 5 % CO for 4 hours. Thereafter, 50 µL of solubilization buffer (R&D Systems, USA) was added to each well to dissolve the colored formazan crystals produced by the reduction of MTT. After 24 hours the optical density was measured at 550 nm using microplate reader (Bio-Rad, USA).

A convenient and efficient route for the preparation of ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylate having an active benzofuran moiety was aimed and developed as shown in Scheme 1. The method involves total four steps (excluding the step preparation of compound **5** from compound **4**) which is very convenient and proceeds through the preparation of new intermediates with reasonably good yields. This method was started from a commercially available 2-bromophenol starting material and other materials which are of commercial grade.



Scheme 1. Synthesis of Ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylate

In the first step, the acetylation of the starting material 2-bromo-4-methoxy-phenol (**1**) was carried out using simple acetylation process by acetic anhydride and acetic acid (1:1) and the acetyl derivative of **1** was nitrated *in situ* to 2-bromo-4-methoxy-5-nitrophenyl acetate **2** simultaneously as a single pot synthesis which was not reported in previous[18]. Later the acetyl nitro derivative **2** was hydrolyzed to 2-bromo-4-methoxy-5-nitrophenol by ester hydrolysis using 1 M lithium hydroxide in THF, methanol, and water (1:1:2). Here the method chosen was hydrolysis by lithium hydroxide instead of sodium and potassium hydroxides because with these strong bases initiated the substitution of bromine by aryl nucleophilic substitution reaction due to the presence of withdrawing nitro group in the *para* position. After completion of 3 hours stirring the mixture was neutralized by 1M HCl followed by workup procedure we got compound **3** with high yield (85 %) and good purity than previously reported. Now the compound **3** was coupled with compound **5** ethyl ester of (4-fluorophenyl)-propynoicacid which in turn is prepared from 1-ethynyl-4-fluorobenzene (**4**) and ethyl chloroformate.

The initial formation of intermediate **6** from **3** by a typical nucleophilic substitution process using potassium carbonate base in acetonitrile solvent refluxed at 110 ^{o}C for 16 hours and successful after several attempts were subjected to *in-situ* intra molecular Heck reaction with palladium acetate, triphenylphosphine (TPP) to obtain compound (**7**). This is the first ever synthetic step for the concurrence of two crucial steps as a single pot with high purity.

Finally, the demethylation of compound **7** was carried using boron trichloride in dichloromethane solvent at 0 ^{o}C, by stirring for 2 hours at room temperature. After completion of the reaction by observing progress in TLC, the reaction mass was treated with sodium bicarbonate to neutralize the acid if any and extracted with ethyl acetate and obtained the product **8** in good yield.

The titled compound now obtained is synthesized in new simplified route and we claim that the method is suitable for synthesis of several other new benzofuran derivatives interesting cytotoxic activities. As described above the key benzofuran moiety in the present investigation is present in several anticancer and antitumor and antiviral compounds[17]. So, the compounds **7** and **8** having benzofuran moiety were tested for their *in vitro* cytotoxicity on HCT116 cell line. We mainly aimed at the activity of these compounds and found that the compound **7** with methyl substitution showed IC_{50} = 95.2 μg/mL and the compound **8** i.e. the final compound showed a good IC_{50} of 8.5 μg/mL on colon cell line.

5. Conclusion

A biologically active benzofuran derivative ethyl 2-(4-fluorophenyl)-5-hydroxy-6-nitrobenzofuran-3-carboxylate was prepared from commercially available 2-bromo-4-methoxy-phenol and *p*-fluorophenylacetylene in good yield. The target molecule was found to possess good cytotoxic activity with IC_{50} of 8.5 μg/mL on HCT 116 cell line.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/OC

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