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Synthesis and antibacterial activity of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carbohydrazide and its Intermediates

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ABSTRACT

Benzofuran derivatives posses a wide range of biological activities. They have been reported to have antitumour , antimicrobial activity. The 2-arylbenzo[b]furan structure is prevalent in a wide variety of biologically active natural and unnatural compounds. The present paper describes the synthesis and antibacterial activity of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carbohydrazide ***8*** *and its related intermediates from commercially available 1,3,5-trimethoxy benzene as staring material in seven steps. The title compound and its intermediates have been screened against four bacterial strains such Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes. It is observed that the title compound, benzo[b]furan carbohydrazide* ***8*** *and benzo[b]furan carboxylic acid* ***6*** *displayed excellent activities.*

Keywords: Benzo[b]furan carbohydrazide, 1,3,5-Trimethoxy benzene, Synthesis, 5-Iodo vanillin, Anti-bacterial activity

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INTRODUCTION

Benzofuran derivatives are now a days an imperative class of organic compounds that occur in a large number of natural products [1] and used in cosmetics [2] and as synthetic pharmaceuticals [3,4]. Benzofuran derivatives posses a wide range of biological activities. They have been reported to have antitumour [5, 6], antimicrobial [7 – 10] activity. The 2-arylbenzo[*b*]furan structure is prevalent in a wide variety of biologically active natural and unnatural compounds [11]. Many 2-arylbenzo[*b*]furan derivatives are well-known to exhibit broad range of biological activities including anticancer [12], antiproliferative [13], anti-inflammatory [14], antiviral [15] and antifungal [16] activities. The investigation of structure-activity relationships for 2-arylbenzo[*b*]furan substituent is still attractive due to variety of biological activities.

Derivatives of benzofuran-2-carboxylic acid are known for exhibiting various pharmacological activities. Such compounds were found to be selective adenosine A2A receptor antagonists [17], anti-inflammatory agents [18], and local anaesthetics [19]. Variously substituted 2-benzofurancarboxylic acid derivatives show selective cytotoxicity against human cancer cell line [20].

Emerging infection diseases and the increasing number of multi-drug resistant microbial pathogen still make the treatment of infection disease an important pressing global problem therefore a substantial research for the discovery and new class of antimicrobial agents is needed.

The search of new high–effective antimicrobial drugs is a very important issue because of the appearance of a large group of antibiotic and antifungal resistant strains [21]. The present paper describes the synthesis and antibacterial activity of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carbohydrazide **8** and its related intermediates from commercially available 1,3,5-trimethoxy benzene as staring material.

MATERIALS AND METHODS

The solvents were purified according to standard procedures prior to use, and all commercial chemicals were used as received. For thin-layer chromatography (TLC) analysis, Merck pre-coated Plates (silica gel 60 F254) were used and spots were visualized with UV light. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography and the eluting solvents are indicated in the procedures. Melting point (mp) determinations were performed by using Mel-temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded in Varian MR-400 MHz instrument. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals were reported as s (singlet), d (doublet), dd (doblet of doblet), t (triplet), q (quartet), m (multiplet) and coupling constants in Hz. The mass spectra were recorded on Agilent ion trap MS. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer.

Experimental methods

Synthesis of 2-iodo-1,3,5-trimethoxybenzene (2)

To a stirred solution of 1,3,5-trimethoxy benzene (10 g, 59.45 mmol) in acetonitrile (60 mL) was added N-Iodosuccinamide (14.70 g, 65.38 mmol) in five portions over a period of 15 min. The reaction mixture was allowed to stir at room temperature for 2 h to obtain white solid. The precipitated solids were filtered, washed with n-Hexane and dried at the pump to afford compound **2**. White solid, Yield: 14.5g, 82%; M.p: 85-87 ^{o}C; IR (KBr): υ_{max} 2964, 2933, 1583, 1467, 1404, 1433, 1342, 1226, 1208, 1159, 1125, 952, 805, 747, 622 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.50 (s, 2 H), 3.86 (s, 6 H), 3.82 (s, 3 H); ESI-MS: m/z, 295.0 (M+1).

Synthesis of (2-(2,4,6-trimethoxyphenyl)ethynyl)trimethylsilane (3)

To a stirred solution of DMF (25 mL) containing compound **2** (5g, 17 mmol), were added sequentially, trimethyl sillyl acetylene (2.85 mL, 20.40 mmol), dichlorobis(triphenylphosphine) palladium (II) (1.2 g, 1.7 mmol), copper iodide (330 mg, 1.7 mmol) and triethylamine (25 mL) were injected through the septum. The reaction mixture was heated for 2 h at 80 ^{o}C in seal tube. The reaction mixture was cooled to room temperature and diluted with diethylether (50 mL), washed with water (4 x 50mL) followed by brine solution, the organic layer was dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to obtain crude compound **3**. Purification was performed by flash chromatography (elluant: 3% EtOAc: Hexane) and an amorphous solid was obtained. Light brown solid, Yield 3.65 g, 81%; M.p: 58-60 ^{o}C; IR (KBr): υ_{max} 2958, 2840, 2149, 1602, 1579, 1494, 1468, 1437, 1415, 1340, 1247, 1228, 1206, 1157, 1130,1057, 1130, 1036, 951, 863 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.06 (s, 2 H), 3.84 (s, 6 H), 3.82 (s, 3 H), 0.26 (s, 9 H); ESI-MS: m/z, 265.0 (M+1).

Synthesis of 2-ethynyl-1,3,5-trimethoxybenzene (4)

To a solution of compound **3** (3.65 g, 15.20 mmol) in methanol (40 mL) was added potassium carbonate (1.7 g, 1.52 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was poured into dichloromethane, washed with brine solution. The organic layer was dried over anhydrous sodium sulfate, and evaporated under reduced pressure to afford crude compound **4**. Crystallisation of the crude compound in n-heptane gave the pure compound **4** as a pale yellow solid. Yield: 1.98 g, 76%; M.p: 119-120 ^{o}C; IR (KBr): υ_{max} 2965, 2937, 2840, 2099, 1582, 1470, 1411, 1345, 1228, 1208, 1191, 1132, 1053, 1032, 808, 784, 664, 621 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.18 (s, 2 H), 3.88 (s, 6 H), 3.83 (s, 3 H), 3.06 (s, 1 H); ^{13}C NMR ($CDCl_3$): δ 55.32 (2C), 55.91, 79.89, 82.41, 85.76, 92.3 (2C),162.44, 165.38 (2C). ESI-MS: m/z, 193.1 (M+1).

Synthesis of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carbaldehyde (5)

To a stirred solution of DMF (10 mL) containing compound **4** (1g, 5.30 mmol) and 5-iodovanillin (1.3 g, 4.45 mmol), were added , dichlorobis(triphenylphosphine) palladium (II) (95 mg, 0.27 mmol), copper iodide (25 mg,

0.13 mmol) and triethylamine (1.25 mL, 8.90 mmol) were injected through the septum in a sealed tube. The reaction mixture was heated for 1.5 h at 70 ^{o}C. The reaction mixture was cooled to room temperature and diluted with diethyl ether (25 mL), the organic layer was separated and washed with water (4 x 20mL) followed by brine solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to obtain crude compound **5**. Purification was performed by flash chromatography (elluant: 10% EtOAc: n-Hexane), to obtain amorphous brown solid. Yield: 1.3 g, 67%; M.p: 101-102 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.00 (s, 1 H), 7.70 (d, *J* = 1.2 Hz, 1H), 7.38 (d, *J* = 1.4 Hz, 1H), 6.87 (s, 1 H), 6.19 (s, 2 H), 4.05 (s, 3H), 3.86 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H); ^{13}C NMR ($CDCl_3$): δ 55.6, 56.2, 55.6, 55.9, 93.2 (2C), 102.8, 104.2, 108.3, 114.9, 125.3, 127.9, 145.5, 150.9, 156.8, 159.4 (2C), 162.7, 191.0; ESI-MS: m/z, 343.2 (M+1).

Synthesis of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carboxylic acid (6)

To a stirred solution of compound **5** (1g, 2.92 mmol) in DMF (20 vol) was added Oxone (14.6 mmol) and stirred at room temperature for 5 h. After completion of the reaction, the reaction mixture was diluted with water and the resultant precipitated compound was filtered and dried under vaccum to obtain pale yellow solid. Yield: 0.84g, 84/%; M.p: 121-122 ^{o}C;

^{1}H NMR (400 MHz, $CDCl_3$): δ 14.02 (s, 1 H), 7.70 (d, *J* = 1.2 Hz, 1H), 7.78 (d, *J* = 1.4 Hz, 1H), 6.87 (s, 1 H), 6.19 (s, 2 H), 4.88 (s, 3H), 3.88 (s, 3 H), 3.80 (s, 3 H), 3.780 (s, 3 H); ESI-MS: m/z, 357.2 (M-1).

Synthesis of methyl 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carboxylate (7)

To a stirred solution of compound **6** (0.84g, 2.45 mmol) in methanol (10 mL) was added catalytic qty; of Conc;H_2SO_4 and refluxed for 10 h. The reaction mixture was diluted with water and extracted with EtOAc (15 mL). The organic layer was washed with water followed brine solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to obtain compound **7**. Yield: 0.70g, 90/%; M.p: 117-119 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1 H), 7.47 (s, 1H), 6.82 (s, 1 H), 6.18 (s, 2 H), 4.05 (s, 3H), 3.93 (s, 3 H), 3.86 (s, 3 H), 3.79 (s, 6 H); ^{13}C NMR ($CDCl_3$): δ 51.5, 55.6 (2C), 56.2, 55.9, 93.2 (2C), 102.8, 104.2, 108.3, 114.9, 124.7, 126.2, 159.4 (2C), 156.8, 144.9, 149.4, 162.7, 166.0; ESI-MS: m/z, 373.2 (M+1).

Synthesis of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carbohydrazide (8)

A mixture of compound **7** (0.5g, 1.30 mmol) and hydrazine hydrate (7.8 mmol) in ethanol (4 mL) was refluxed for 12 h. The reaction mixture was diluted with excess of EtOAc, the organic layer was washed with water followed by brine solution. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and evaporated to obtain off-white solid. Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (br.s, 1H), 7.75 (s, 1 H), 7.52 (s, 1H), 6.86 (s, 1 H), 6.15 (s, 2 H), 4.05 (s, 3H), 3.42 (br.s, 2 H), 3.93 (s, 3 H), 3.86 (s, 3 H), 3.84 (s, 3 H); ^{13}C NMR ($CDCl_3$): δ 55.6 (2C), 55.9, 56.2, 93.2 (2C), 102.8, 104.2, 105.9, 112.5, 124.9, 145.1, 148.5, 156.8, 159.4 (2C), 162.7, 164.9; ESI-MS: m/z, 372.2 (M+1).

BIOLOGICAL ASSAY

All the synthesized intermediates and the title compound **8** were dissolved in dimethyl sulphoxide at 25 µg/mL concentration. Compound **8** and its related intermediates were tested against two Gram negative strains viz., i) *Escherichia coli* (MTCC443), *(ii) Pseudomonas aeruginosa* (MTCC424) and two Gram positive strains viz., (iii) *Staphylococcus aureus* (MTCC96) strains *iv) Streptococcus pyogenes* (MTCC442) using agar well diffusion method according to the literature protocol [22-24]. The composition of nutrient agar medium was Yeast extract (5 g), NaCl (10 g), Bactotryptone (10 g), final pH 7.4. After 18 h the exponentially growing cultures of the four bacteria in nutrient broth at 37 ^{o}C were diluted in sterile broth. From each of these diluted cultures, 1mL was added to 100 mL sterilized and cooled nutrient agar media to give a final bacterial count of 1 x 10^6 cell/ml. The plates were set at room temperature and later dried at 37 ^{o}C for 20h. Paper discs (6mm, punched from whatmann no 41 paper) were ultraviolet sterilized and used for the assays. Discs were soaked in different concentration of the test solution and placed on the inoculated agar media at regular intervals of 6-7 cm, care was taken to ensure that excess solution was not on the discs. The plates were incubated at 37 ^{o}C in an inverted fashion. Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicates.

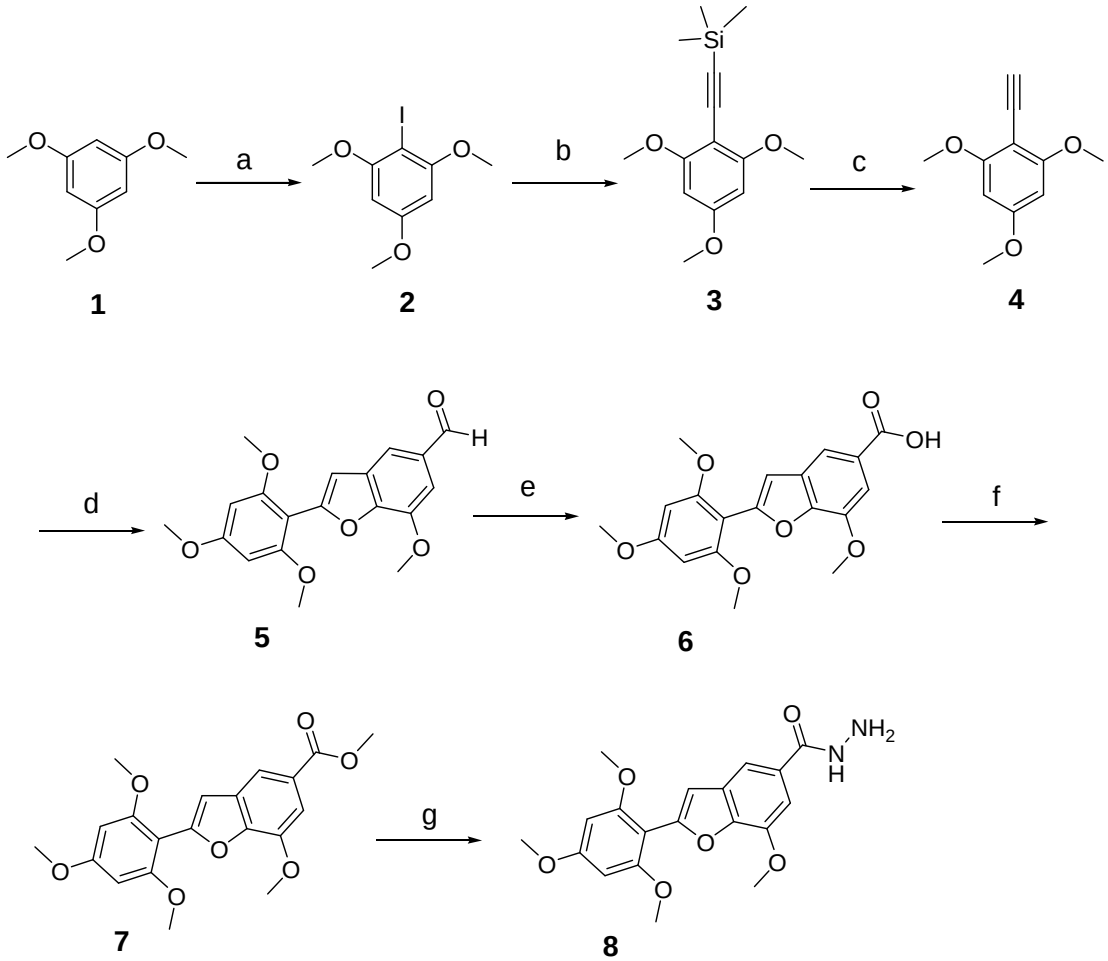
RESULTS AND DISCUSSIONS

Chemistry

The title compound and its related intermediates described in this paper were prepared according to the synthetic **Scheme 1**. The structures of the synthesized compounds were confirmed by ^{1}H NMR and Mass data. Iodination of 1,3,5-trimethoxy benzene **1** was carried out using N-Iodosuccinamide in acetonitrile at room temperature gave the iodinated compound **2**, which upon coupling with TMS-acetylene, using the Sonogashira protocol $Pd(PPh_3)_2Cl_2/CuI/Et_3N$, afforded sillylated compound **3**. De-sillylation of compound **3** was done using K_2CO_3 in methanol to produce phenyl acetylene derivative **4**. Compound **4** was reacted with 5-iodovanillin in presence of $Pd(PPh_3)_2Cl_2/CuI/Et_3N$, in DMF to furnish benzofuran aldehyde **5**. Benzo[b]furan aldehyde **5** was oxidized to benzo[b]furan carboxylic acid **6** in presence of oxone in DMF. Esterification of benzo[b]furan carboxylic acid **6** in methanol in presence of cat; H_2SO_4 resulted in corresponding methylester derivative **7**. Reaction of ester **7** with hydrazine hydrate resulted in the formation of benzo[b]furan carbohydrazide **8**.

Anti-bacterial activity

The antibacterial activity result (**Table 1**) revealed that the title compound and its intermediates **1 – 8**, showed varying pattern of inhibition against the tested microorganisms. In general, it is observed that benzo[b]furan carbohydrazide **8** and benzo[b]furan carboxylic acid **6** displayed excellent activity against, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*, while compounds **4** showed good activity and the compound **3** exhibited moderate activity. The remaining compounds such as **1,2, 5** and **7** were found to be inactive against all the tested bacterial strains.



Scheme 1. Synthesis of Benzo[b]furan carbozide 8

Experimental Conditions: a) N-Iodosuccinamide, acetonitrile, r.t., 2 h; b) TMS—acetylene, $Pd(PPh_3)_2Cl_2$, CuI, triethylamine, DMF, 80 ^oC, 2 h; c) K_2CO_3, MeOH, r.t, 16 h; d) 5-iodovanillin, $Pd(PPh_3)_2Cl_2$, CuI, triethyl amine, DMF, 70 ^oC, 1.5 h; e) Oxone, DMF, r.t., 5 h; f) MeOH, cat; H_2SO_4, reflux, 10 h; g) Hydrazine-Hydrate, EtOH, reflux, 12 h.

Table-1: Antibacterial Activity of Compounds 1-8 (Concentration Used 25 µg/mL of DMSO).

Compound No.	*Gram negative*		*Gram positive*	
	E.Coli ***MTCC 443***	*P.aeruginosa* ***MTCC 424***	*S.Aureus* ***MTCC 96***	*S.Pyogenes* ***MTCC 442***
	Zones of Inhibition of compounds 6a –6o in mm			
1	--	--	--	--
2	--	--	--	--
3m	18	17	21	17
4g	22	20	25	20
5	--	--	--	--
6e	26	21	27	23
7	--	--	--	--
8e	27	22	26	21
Standard Drug Norfloxacin (25 µg/mL of DMSO)	25	19	25	19

"—" : no activity

CONCLUSION

In conclusion, the present paper describes the synthesis of 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carbohydrazide **8** from commercially available 1,3,5-trimethoxy benzene as staring material in seven steps. The title compound and its intermediates have been screened against four bacterial strains such *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. It is observed that title compound, benzo[b]furan carbozide **8** and benzo[b]furan carboxylic acid **6** displayed excellent activities while compounds **4** showed good activity and the compound **3** exhibited moderate activity.

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