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

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]. Various substituted 2-benzofuran carboxylic 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 for 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 starting 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.

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Experimental methods

Synthesis of 2-ido-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-iodosuccinimide (14.70 g, 65.38 mmol) in five portions over a period of 15 min. The reaction mixture was stirred 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: 14.5 g, 82%; M.p: 85-87 °C; IR (KBr): \( \nu_{\text{max}} \) 2964, 2933, 1583, 1467, 1404, 1433, 1342, 1226, 1208, 1159, 1125, 952, 805, 747, 622 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 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)ethyltri methylsilane (3)

To a stirred solution of DMF (25 mL) containing compound 2 (5 g, 17 mmol), were added sequentially, trimethylsilyl 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 °C in seal tube. The reaction mixture was cooled to room temperature and diluted with diethyl ether (50 mL), washed with water (4 x 50mL) followed by brine solution, the organic layer was dried over anhydrous sodium sulfate, 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: 1.98 g, 76%; M.p: 119-120 °C; IR (KBr): \( \nu_{\text{max}} \) 2958, 2840, 2149, 1602, 1579, 1494, 1468, 1437, 1415, 1340, 1247, 1228, 1206, 1157, 1130, 1036, 951, 863 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 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 7-methoxy-2-(2,4,6-trimethoxyphenyl)benzofuran-5-carboxaldehyde (5)

To a stirred solution of DMF (10 mL) containing compound 4 (1.0 g, 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, and new class of antimicrobial agents is needed.

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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 °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 20 mL) 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 (eluent: 10% EtOAc: n-Hexane), to obtain amorphous brown solid. Yield: 1.3 g, 67%; M.p: 101-102 °C; 1H NMR (400 MHz, CDCl3): 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); 13C NMR (CDCl3): 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: 0.84 g, 84%; M.p: 121-122 °C;

1H NMR (400 MHz, CDCl3): 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-I).

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:H2SO4 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 with EtOAc (15 mL). 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 °C; 1H NMR (400 MHz, CDCl3): 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); 13C NMR (CDCl3): 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-carboxyhydrazide (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 Na2SO4, filtered and evaporated to obtain off-white solid. Yield: 78%. 1H NMR (400 MHz, CDCl3): δ 7.89 (br.s, 1 H), 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); 13C NMR (CDCl3): 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).

BIOLGICAL ASSAY
All the synthesized intermediates and the title compound 8 were dissolved in dimethyl sulphoxide at 25 µM concentration. Compound 8 and its related intermediates were tested against two Gram negative strains viz., (i) *Escherichia coli* (MTCC443), (ii) *Pseudomonas aeruginosa* (MTCC242) 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 °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 10^8 cell/mL. The plates were set at room temperature and later dried at 37 °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 °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$_3$)$_2$Cl$_2$/CuI/Et$_3$N, afforded silylated compound 3. De-silylation of compound 3 was done using K$_2$CO$_3$ in methanol to produce phenyl acetylene derivative 4. Compound 4 was reacted with 5-iodovanillin in presence of Pd(PPh$_3$)$_2$Cl$_2$/CuI/trimethylamine 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$_2$SO$_4$ resulted in corresponding methylester derivative 7. Reaction of ester 7 with hydrazine hydrate resulted in the formation of benzo[b]furan carbohydraze 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 carbohydraze 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 carbohydraze 8**

**Experimental Conditions:**
a) N-Iodosuccinamide, acetonitrile, r.t., 2 h; b) TMS—acetylene, Pd(PPh$_3$)$_2$Cl$_2$, Cui, triethylamine, DMF, 80 ºC, 2 h; c) K$_2$CO$_3$, MeOH, r.t., 16 h; d) 5-iodovanillin, Pd(PPh$_3$)$_2$Cl$_2$, Cui, triethyl amine, DMF, 70 ºC, 1.5 h; e) Oxone, DMF, r.t., 5 h; f) MeOH, cat; H$_2$SO$_4$, 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).

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<tr>
<th>Compound No.</th>
<th>Gram negative</th>
<th>Gram positive</th>
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<td>E.Coli MTCC 443</td>
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“--” : 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, benzofuran carbozide 8 and benzofuran carboxylic acid 6 displayed excellent activities while compounds 4 showed good activity and the compound 3 exhibited moderate activity.

REFERENCES