Potential anti-cancer performance of chitosan-based \( \beta \)-ketosulfone derivatives

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PUBLIC INTEREST STATEMENT

The current work is dealing with the synthesis and characterization of a new series of chemically modified chitosan and evaluates its anti-cancer activity against three types of cancer cell lines (colon carcinoma, liver hepatocellular carcinoma, and breast carcinoma). The structures were determined by elemental and spectra tools. The products were also characterized by various characterization techniques. The synthesized compounds showed a significant biological screening against Gram-positive, Gram-negative bacteria and fungi. Among all tested products, \((\text{CsB-}\beta\text{-KS})_3\) displayed higher efficiencies toward all three types of cancer under investigation with considerable low concentrations.
Abstract: A series of chitosan-based β-ketosulfone derivatives (CsB-β-KS) were synthesized, characterized, and evaluated as anti-cancer agents against three types of cancer cell lines, including the colon carcinoma (HCT), liver hepatocellular carcinoma (HEPG2), and breast carcinoma (MCF-7) cell lines. Before product formation, the β-ketosulfone derivatives, 1-(4-halophenyl)-2-(phenylsulfonyl)ethanone, were synthesized by the reaction of phenacyl halide with sodium benzene sulfinate. The (CsB-β-KS) α-e derivatives were synthesized by chemical modification of chitosan (Cs) with freshly prepared p-halo-β-ketosulfone derivatives in a mildly acidic aqueous solution. Various loading percentages, 5%, 10%, 15%, and 20%, of the p-halo-β-ketosulfone derivative (by weight) with respect to the Cs weight were evaluated. The chemical structures were confirmed by variable elemental and spectral analyses, including FT-IR, $^1$H-NMR, $^{13}$C-NMR, and mass spectrometers. The (CsB-β-KS)α-e derivatives were also characterized by various techniques, such as FT-IR, $^1$H-NMR, XRD, FE-SEM, and thermal analyses. FT-IR spectroscopy and XRD confirmed the formation of these products. Moreover, the XRD results proved the strong interactions between the organic substituent and the Cs host molecule. All (CsB-β-KS)α-e derivatives showed similar thermal stabilities in three degradation steps. Among these derivatives, (CsB-β-KS)α3 showed the highest thermal stability. The synthesized compounds showed significant biological screening against Gram-positive and Gram-negative bacteria and fungi. Among the tested products, (CsB-β-KS)α3 displayed high efficiencies toward the three types of cancer cell lines under investigation with low concentrations. The ranking of the anti-cancer activity was (CsB-β-KS)α3 > (CsB-β-KS)α2 > (CsB-β-KS)α1.

Subjects: Environment & Agriculture; Bioscience; Physical Sciences

Keywords: chitosan; β-ketosulfone; chemical modification; colon; liver; breast; anti-cancer

1. Introduction
In industry and research, the use of biopolymers has increased over the last few years because of their low cost, non-toxicity, environmentally friendly processing, and biocompatibility. Chitosan (Cs) was selected for investigation in this study because of its wide availability, low cost, nontoxicity, biodegradability, and unique structure (Kumar, 2000; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; Sreenivasan, 1996). Cs is prepared from chitin, which is harvested from shrimp shells, crab, or fungal mycelia. Cs is easily modified and considered as a good source for nanoparticles, membranes, and fibrous materials. Moreover, it shows excellent chemical and biological properties owing to the activity of the two reactive groups, which allow a chemical modification of the Cs with organic and inorganic compounds. Cs-based sulfone derivatives can affect the treatment of a cancer patient. The five-year survival rate for cancer patients has remained ~50% for the last several decades. Chemotherapy with 5-fluoro-uracil (5-FU), docetaxel, and cisplatin, for oral cancer, is associated with serious side reactions and effects (Andreadis et al., 2003); therefore, intensive research has been performed on the discovery of anticancer agents from natural sources with minimal toxicity on normal cells. The biopolymer Cs is a derivative of chitin, which is obtained by the deacetylation of its precursor chitin (Wimardhani, Suniarti, Freisleben, Wanandi, & Ikeda, 2012). Several reports have shown that low-molecular-weight Cs (LMWC) exerts a cytotoxic effect on oral cancer cells (Sreenivasan, 1996). Identification of the molecules activated by Cs would be useful for understanding the mechanisms responsible for the cytotoxic effects of Cs on cancer cells. The biopolymer Cs induces apoptosis and activates caspase-3 (Sugano et al., 1980), as well as activates the extrinsic apoptosis pathway through activation of caspase-8 (Takimoto et al., 2004). Necrosis is the mechanism responsible for cell death when cancer cells are exposed to Cs nanoparticles (Qi, Xu, Li, Jiang, & Han, 2005). Inhibition of the tumor cell cycle related to p21/CIP, p27/KIP, and the proliferating cell
nuclear antigen (PCNA) has also been reported (Lin et al., 2007). The use of Cs derivatives in drug delivery and gene therapy was discovered in the early 19th century. Huang et al. and others (Hu, Chen, Zhao, Yuan, & Du, 2013; Huang et al., 2012; Li, Yawata, & Honke, 2014; Termsarasab et al., 2013; Xu, Wang, Li, & Wang, 2014; Zhu et al., 2013) studied many derivatives of Cs oligosaccharide as potential carriers for intracellular delivery of anticancer agents. Conversely, sulfone derivatives are a versatile class of compounds (Gunda & Sz'oke, 1998; Simpkin, 1993), which have various applications, such as organic synthesis, pharmaceutical fields, and different areas (Bandarage et al., 2008; Chen et al., 2007; Helmy, Fahmy, & Sabry, 2008; Jaishankar et al., 2008; Steert et al., 2007; Tozkoparan, Küpeli, Yeşilada, & Ertan, 2007; Xiang et al., 2007). Thus, sulfones have received much attention and an increased effort to develop new approaches for a variety of compounds incorporating phenylsulfonyl moiety for biological screening. Many sulfonyl compounds have been synthesized bearing biologically active moieties (Al-Said, Ghorab, & Nissan, 2012). Sulfated derivatives of Cs inhibit the proliferation of the human breast cancer cell lines, MDA-MB-231 and MCF-7, using dextrorubicin as a reference drug, and potential anticancer activity has been exhibited. The PI3K/Akt/m-TOR signaling pathways are an important class in cancer biology owing to their involvement in the regulation of cell growth, proliferation, survival, and angiogenesis. Therefore, they offer an attractive therapeutic target for tumor growth inhibition (Welker & Kulik, 2013). A series of 2-arylamino-3-(arylsulfonyl) quinoxalines was synthesized as PI3Kα inhibitors by Wu et al. in 2011 (Wu et al., 2011). Most of the targeted compounds of this series displayed favorable cytotoxicities and excellent cellular potencies. Another series of sulfonyl-morpholinopyrimidines as selective m-TOR kinase inhibitors has been synthesized. To increase the m-TOR potency and selectivity over PI3K, two libraries of compounds were prepared by changing the pyridyl moiety and by using different substituents on sulfoxone moiety. The introduction of 5-indole and urea moiety increases the m-TOR inhibition in both enzyme and cellular assays (Finlay et al., 2012; Lium et al., 2012). This study aimed to synthesize and characterize a series of Cs-based β-ketosulfone derivatives, (CsB-β-KS)a-e. A complete biological screening was performed against a number of Gram-positive and Gram-negative bacteria and fungi in order to determine the best performance. In addition, the synthesized products were evaluated as anti-cancer agents against three types of cancer cell lines, including HCT, HEPG2, and MCF-7.

2. Experimental

2.1. Materials and equipment

Cs with >75% deacetylation and a high average molecular weight of 800–2000 cP was purchased from Sigma Aldrich Co., Ltd. Phenacyl bromide derivatives were purchased from Sigma Aldrich Co., Ltd. Sodium benzene sulfinate was also purchased from Sigma Aldrich Co., Ltd. Dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) were purchased from Duksan Chemical Co., Ltd. (Ansan, Korea). All materials in this study were used as received, without further purification.

All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded in potassium bromide discs on Pye Unicam SP 3–300 and Shimadzu FT-IR 8101 PC infrared spectrophotometers. The NMR spectra recorded on a Varian Mercury VXK-400 NMR spectrometer (400 MHz) and Bruker-500 NMR spectrometer (500 MHz) were carried out in deuterated chloroform (CDCl3), deuterated dimethylsulfoxide (DMSO-d6), and deuterated water (D2O). The chemical shifts were related to that of the solvent. The mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. The XRD patterns were performed for the nanoparticles and composites in the 2θ range from 10° to 80° using a Bruker diffractometer (Bruker D8 advance target). The patterns were carried out with copper Ka1 and a monochromator (l = 1.5405 Å) at 40 kV and 40 mA. The TGA and DTG curves were recorded with a TA instrument apparatus model TGA-Q500 using a heating rate of 10°C min⁻¹ in a nitrogen atmosphere. The average masses of the samples were 5 mg. The morphological properties of the new composites were analyzed by a field-emission scanning electron microscope (FE-SEM) on a JEOL model JSM-7600F microscope.
2.2. Preparation of the 1-(4-aryl)-2-(phenylsulfonyl)ethanone derivatives

The sulfone-based derivatives were prepared according to the procedures in the reported literature (Curti, Laget, Ortiz Carle, Gellis, & Vanelle, 2007; Takahashi, Mamiya, & Wakao, 1986) as follows. Sodium benzene sulfinate (2 mmol) was added in a dropwise manner to an ethanol solution of phenacyl bromide derivatives (2 mmol). The reaction mixture was heated under reflux for 6 h. After cooling, the reaction mixture was treated with water, and the solid product obtained was filtered and crystallized from the appropriate solvent. The physical and spectral characteristic data of the 1-(4-aryl)-2-(phenylsulfonyl)ethanone derivatives are shown below.

2.2.1. 1-(4-Bromophenyl)-2-(phenylsulfonyl)ethanone (a)

Yield (92%); m.p. 144–146 °C [Lit m.p. 145–147 °C] (Sreedhar, Rawat, & Regioselective Catalyst, 2012); IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1691 (C = O), 1181, 1391 (SO$_2$); $^1$H-NMR (CDCl$_3$): $\delta$ = 4.68 (s, 2H, CH$_2$), 7.54 (t, 2H, J = 7.8 Hz), 7.61–7.67 (m, 3H), 7.80–7.86 (m, 4H); $^{13}$C-NMR (CDCl$_3$): $\delta$ = 63.60, 128.33, 130.07, 130.81, 132.28, 134.42, 134.46, 138.54, 187.10; MS (m/z): 339 (M$^+$); Anal. Calcd for C$_{14}$H$_{11}$BrO$_3$ S (339.20): C, 49.57; H, 3.27; S, 9.45%. Found: C, 49.52; H, 3.22; S, 9.43%.

2.2.2. 2-(Phenylsulfonyl)-1-p-tolyl ethanone (b)

Yield (90%); m.p. 128–130 °C [Lit m.p. 127–129°C] (Yang, Li, Li, Peng, & Gu, 2012); IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1691 (C = O), 1181, 1393 (SO$_2$); $^1$H-NMR (CDCl$_3$): $\delta$ = 2.41 (s, 3H, CH$_3$), 4.70 (s, 2H, CH$_2$), 7.25 (d, 2H, $J$ = 8.2 Hz), 7.51 (t, 2H, $J$ = 7.6 Hz), 7.65 (dd, 1H, $J$ = 10.7 and 4.2 Hz), 7.81–7.87 (m, 4H); $^{13}$C-NMR (CDCl$_3$): $\delta$ = 21.82, 63.47, 128.62, 129.20, 129.50, 129.61, 133.55, 134.21, 138.78, 145.66, 187.48; MS (m/z): 274 (M$^+$); Anal. Calcd for C$_{15}$H$_{12}$O$_3$ S (274.33): C, 65.67; H, 5.14; S, 11.69%. Found: C, 65.63; H, 5.18; S, 11.73%.

2.2.3. 1-Phenyl-2-(phenylsulfonyl)ethanone (c)

Yield (85%); m.p. 89–91 °C [Lit m.p. 90–92 °C] (Yang et al., 2012); IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1691 (C = O), 1181, 1391 (SO$_2$); $^1$H-NMR (CDCl$_3$): $\delta$ = 4.73 (s, 2H, CH$_2$), 7.46 (dd, 2H, $J$ = 10.4 and 4.8 Hz), 7.53 (dd, 2H, $J$ = 10.4 and 4.9 Hz), 7.60–7.65 (m, 2H), 7.88–7.92 (m, 4H); $^{13}$C-NMR (CDCl$_3$): $\delta$ = 63.60, 128.33, 130.07, 130.81, 132.28, 134.42, 134.46, 138.54, 187.10; MS (m/z): 260 (M$^+$); Anal. Calcd for C$_{15}$H$_{13}$O$_3$ S (260.31): C, 64.60; H, 4.65; S, 12.32%. Found: C, 64.54; H, 4.33; S, 12.37%.

2.2.4. 1-(4-Chlorophenyl)-2-(phenylsulfonyl)ethanone (d)

Yield (90%); m.p. 135–137 °C [Lit m.p. 136–138 °C] (Yang et al., 2012); IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1691 (C = O), 1181, 1391 (SO$_2$); $^1$H-NMR (CDCl$_3$): $\delta$ = 4.69 (s, 2H, CH$_2$), 7.45–7.54 (m, 2H), 7.67 (t, 2H, $J$ = 7.8 Hz), 7.83–7.85 (m, 5H); $^{13}$C-NMR (CDCl$_3$): $\delta$ = 63.61, 128.57, 129.27, 129.32, 130.78, 134.07, 134.41, 138.56, 141.20, 186.87; MS (m/z): 294 (M$^+$); Anal. Calcd for C$_{15}$H$_{12}$ClO$_3$ S (294.75): C, 57.05; H, 3.76; S, 10.88%. Found: C, 57.10; H, 3.73; S, 10.81%.

2.2.5. 1-(4-Fluorophenyl)-2-(phenylsulfonyl)ethanone (e)

Yield (88%); m.p. 120–122 °C [Lit m.p. 121–123 °C] (Xuan, Feng, Chen, Lu, & Xiao, 2014); IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1691 (C = O), 1181, 1391 (SO$_2$); $^1$H-NMR (CDCl$_3$): $\delta$ = 4.69 (s, 2H, CH$_2$), 7.14 (dd, 2H, $J$ = 10.8 and 4.8 Hz), 7.54 (t, 2H, $J$ = 7.6 Hz), 7.66 (dd, 1H, $J$ = 10.8 and 4.2 Hz), 7.88–7.99 (m, 4H); $^{13}$C-NMR (CDCl$_3$): $\delta$ = 63.61, 116.05, 116.27, 128.56, 129.30, 132.21, 132.25, 132.30, 133.48, 138.61, 165.23, 167.79, 186.42; MS (m/z): 279 (M$^+$); Anal. Calcd for C$_{15}$H$_{12}$FO$_3$ S (279.30): C, 60.42; H, 3.98; S, 11.52%. Found: C, 60.45; H, 3.92; S, 11.55%.

2.3. Synthesis of Cs-based 8-ketosulfone (CsB-8-KS)a–e

In a typical procedure, (CsB-8-KS)a–e was synthesized as follows. A mixture of 1 g of powder Cs and previously prepared 1-(4-aryl)-2-(phenylsulfonyl)ethanone derivatives were stirred in H$_2$O:AcOH (9:1) at an ambient temperature. Each derivative was chemically treated with Cs four times using four different weight ratios (5%, 10%, 15%, and 20% with respect to the Cs weight). The reaction mixture was subjected to a constant stirring until the complete disappearance of the starting material (8-ketosulfone), which was monitored by TLC. The reaction was complete within 4–6 h for all compounds. Finally, the solvent evaporated, and the residue product was dried and
analyzed. The final products contained the symbols \((\text{CsB-β-KS})_{a1-4}\), \((\text{CsB-β-KS})_{b1-4}\), \((\text{CsB-β-KS})_{c1-4}\), \((\text{CsB-β-KS})_{d1-4}\), and \((\text{CsB-β-KS})_{e1-4}\) for the bromophenyl-, tolyl-, phenyl-, chlorophenyl-, and flour-ophenyl derivatives, respectively. The spectral data for the \((\text{CsB-β-KS})_{a-e}\) derivatives are given as follows.

2.3.1. \((\text{CsB-β-KS})_{a3}\)

IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3305 (OH stretching), 2953 (C-H stretching), 1630 (amide), 1550 (OH bending), 1085 (C-O stretching), 1100–1400 (SO$_2$); $^1$H-NMR (CDCl$_3$/D$_2$O): \(\delta\) 1.9–3.2 (H2 protons of glucosamine), 4.6–4.8 (anomeric protons), 4.84 (sulfone CH$_2$ protons), \(\delta\) 7.26 (t, 1H), 7.56 (t, 2H), 7.62–7.67 (m, 5H), 7.80–7.82 (d, 2H), 7.86 (d, 2H); $^{13}$C-NMR (CDCl$_3$/D$_2$O): \(\delta\) 55.87, 60.06, 60.35, 69.64, 70.08, 71.80, 74.80, 97.53, 97.76, 128.57, 129.32, 130.81, 132.29, 134.44.

2.3.2. \((\text{CsB-β-KS})_{b3}\)

IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3305 (OH stretching), 2953 (C-H stretching), 1630 (amide), 1550 (OH bending), 1085 (C-O stretching), 1100–1400 (SO$_2$); $^1$H-NMR (CDCl$_3$/D$_2$O): \(\delta\) 1.99–3.17 (H2 protons of glucosamine), 3.50–4.02 (non-anomeric protons of the Cs residue), 4.68–4.88 (anomeric protons), 7.25–7.27 (d, 2H), 7.51–7.55 (t, 2H), 7.63–7.65 (t, 1H), 7.82–7.83 (d, 2H), 7.87–7.89 (d, 2H); $^{13}$C-NMR (CDCl$_3$/D$_2$O): \(\delta\) 22.82, 22.23, 54.02, 54.37, 54.89, 56.72, 60.38, 67.95, 68.77, 69.66, 69.77, 69.97, 70.10, 71.65, 74.38, 74.82, 128.58, 129.19, 129.48, 132.39, 134.18, 174.57, 174.70, 174.83.

2.3.3. \((\text{CsB-β-KS})_{c3}\)

IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3305 (OH stretching), 2953 (C-H stretching), 1630 (amide), 1550 (OH bending), 1085 (C-O stretching), 1100–1400 (SO$_2$); $^1$H-NMR (CDCl$_3$/D$_2$O): \(\delta\) 2.06–3.23 (H2 protons of glucosamine), 3.52–3.98 (non-anomeric protons of the Cs residue), 4.74–4.88 (anomeric protons), 7.40 (m, 10H, aromatic protons). $^{13}$C-NMR (CDCl$_3$/D$_2$O): \(\delta\) 22.04, 54.27, 54.38, 55.90, 56.74, 60.11, 60.39, 67.96, 68.03, 69.68, 69.79, 69.98, 70.09, 71.67, 71.82, 74.83, 76.43, 88.95, 89.20, 92.65, 92.78, 97.53, 97.78, 129.22.

2.3.4. \((\text{CsB-β-KS})_{d3}\)

IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3305 (OH stretching), 2953 (C-H stretching), 1630 (amide), 1550 (OH bending), 1085 (C-O stretching), 1100–1400 (SO$_2$); $^1$H-NMR (CDCl$_3$/D$_2$O): \(\delta\) 1.97–3.11 (H2 protons of glucosamine), 3.45–3.46 (non-anomeric protons of the Cs residue), 4.71–4.89 (anomeric protons), 7.43–7.85 (m, 9H); $^{13}$C-NMR (CDCl$_3$/D$_2$O): \(\delta\) 55.08, 59.98, 60.29, 69.58, 69.79, 70.02, 71.74, 128.57, 129.28, 130.79, 134.07, 134.41.

2.3.5. \((\text{CsB-β-KS})_{e3}\)

IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3305 (OH stretching), 2953 (C-H stretching), 1630 (amide), 1550 (OH bending), 1085 (C-O stretching), 1100–1400 (SO$_2$); $^1$H-NMR (CDCl$_3$/D$_2$O): \(\delta\) 2.00–3.20 (H2 protons of glucosamine), 3.53–4.02 (non-anomeric protons of the Cs residue), 4.69–4.84 (anomeric protons), 7.22–7.98 (m, 9H); $^{13}$C-NMR (CDCl$_3$/D$_2$O): \(\delta\) 21.93, 22.15, 22.22, 53.96, 54.17, 54.29, 55.80, 59.98, 60.29, 69.58, 70.02, 71.74, 88.89, 89.14, 90.61, 92.60, 92.73, 94.78, 97.20, 97.48, 97.71, 102.30, 116.33, 128.45, 129.18, 132.17, 174.52.

2.4. **Antimicrobial assays**

2.4.1. **Test organisms**

Two Gram-positive bacteria [*Streptococcus pneumonia* (RCMB010010) and *Bacillus subtilis* (RCMB 010067)], two Gram-negative bacteria [*Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052)], and two fungi [*Aspergillus fumigatus* (RCMB 02568) and *Candida albicans* (RCMB 05036)] were obtained from The Biology Department, National Research Center, Cairo, Egypt.
2.4.2. Antimicrobial activity

The Cs derivatives (CsB-β-KS)a-e under investigation were tested for antimicrobial activity against the above-mentioned test organisms. The test organisms were cultured on nutrient agar on Petri dishes for 72 h at 35°C (diffusion agar technique). The antimicrobial activities against the tested organisms were evaluated by measuring the diameter of their inhibition zone. The hole-plate diffusion method was used. Six equidistant (1-cm diameter) holes were made using a sterile cork borer in a malt extract agar and nutrient agar sterile plates (10 × 10 cm), which were previously seeded with tested fungal and bacterial isolates. The holes were filled with 100 mL of a 5-mg/ml concentration of each of the synthesized compounds after completely dissolving in DMSO/0.1 M AcOH. The control holes were filled with DMSO/0.1 M AcOH solvent. The plates were placed in a cooled incubator at 4 (± 2) °C for 1 h and then incubated at 37 (± 2) °C for the bacterial isolates and incubated at 28 (± 2) °C for the fungal isolates. The inhibition zones that developed owing to the active ingredients were measured after 24–48 h of incubation time. Amphotericin B was used as a standard antifungal agent, while ampicillin and gentamycin were used as standard antibacterial agents.

2.5. Minimum inhibitory concentration (MIC) assays

The MIC was determined by a serial dilution technique described by Irobi et al. (Irobi, Moo-Young, & Anderson, 1996). An initial maximum concentration of 500 mg/mL of DMSO/0.1MAcOH solvent of the synthesized compounds was used, and then, the concentration was reduced by successive twofold dilutions of that stock solution using a calibrated micropipette. The MIC of the sample was determined by inoculation of their serial dilutions with the test organisms and the measurement of the inhibition zones using the diffusion agar technique. The MIC was expressed as the lowest concentration inhibiting the test organism's growth (Curti et al., 2007; Irobi et al., 1996).

2.6. Anti-cancer activity

The Mammalian cell lines: HCT, HEPG2, and MCF-7, were obtained from the VACSERA Tissue Culture Unit, Egypt. In order to compare the cytotoxic effects on tumor cells after the treatment with the modified Cs derivatives, we also included treatments in a normal cell line (Chinese hamster lung fibroblasts; V79) which obtained from Sigma-Aldrich Co. The cellular toxicity of the modified Cs derivatives was assessed in vitro by the Skehan et al. assay. Multidose experiments on the HCT, HEPG2, and MCF-7 were performed for (CsB-β-KS)a3, (CsB-β-KS)d2, and (CsB-β-KS)d3, which were the most active compounds against the test organisms. Different concentrations (0.0, 5.0, 12.5, 25.0, and 50 µg/mL) of the selected modified Cs derivatives were used. One hundred microliters of the minimum essential medium (MEM) were dispensed in 96-well flat-bottomed plates. The cells remained in the 96-multiwell plate (10⁴ cell/well) for 24 h before treatment with the compounds to allow attachment of the cell to the wall of the plate. Then, the compounds were added to the wells in triplicate for each individual dose. The monolayer cells were incubated with the compounds for 48 h at 37°C in an atmosphere of 5% CO₂. After 48 h, the cells were fixed, washed, and stained with sulforhodamine-B stain. The excess stain was washed with acetic acid, and the attached stain was recovered with Tris-EDTA buffer. The color intensity was measured in an ELISA reader. The relationship between the surviving fraction and compound concentration was plotted to obtain the survival curve of each tumor cell line to estimate IC₅₀. Doxorubicin, a cancer chemotherapeutic drug, served as positive control while DMSO, the solvent, served as a negative control.

3. Results and discussion

3.1. Chemistry of the Cs-based 6-ketosulfone (CsB-6-KS)a-e

Cs derivatives are widely used in different fields, and in most cases, they are prepared by a chemical modification process using variable synthetic strategies as reported in the literature (Kumar, 2000; Mourya & Inamdar, 2008). Among the derivatives, one derivative can differentiate between specific reactions involving the -NH₂ group at the C-2 position or nonspecific reactions of the hydroxyl (-OH) groups at the C-3 and C-6 positions (esterification and etherification).
Takahashi et al., 1986). The reactive amino group on the backbone enables Cs to create several chemical modifications, which facilitates its usage in different applications. Since Cs is produced by chitin deacetylation, which is widely distributed in nature and is the second most abundant biopolymer, most of the derivatives are obtained from highly deacetylated chitin (Dorman & Deans, 2000). However, β-ketosulfones are synthesized before the chemical modification of Cs. The synthesis of β-ketosulfones is carried out in numerous ways. The most accessible and common synthetic method used to synthesize β-ketosulfones is the alkylation of metallic aryl sulfinate with α-halo ketones. Therefore, 4-halophenacyl bromide derivatives react with the sodium salt of sulfenic acid under reflux conditions to afford the corresponding β-ketosulfone derivatives with a high yield, as shown in Figure 1. The structure of the synthesized β-ketosulfone derivatives was confirmed by the spectroscopic data that was in agreement with the proposed structure given in the experimental section.

The synthesis of the Cs-based β-ketosulfone derivatives (CsB-β-KS)a-e was carried out via the condensation of the previously synthesized ketosulfones (five derivatives) with pure Cs in different loading percentages for each derivative. Thus, (CsB-β-KS)a-e was synthesized by stirring a mixture of Cs and 1-(4-aryl)-2-(phenylsulfonyl)ethanone derivatives in H₂O:AcOH as a mixed solvent at room temperature, as shown in Figure 2. The synthetic procedures are given in the experimental section. The spectroscopic tools confirmed the incorporation of the sulfone groups into the Cs backbone.

The ¹H-NMR and ¹³C-NMR data recorded in the mixture of CDCL₃ and D₂O, and the results showed the presence of the β-ketosulfone moiety in the region of the aromatic frequencies of the NMR spectra of the prepared samples. There was a significant change in the spectra of the Schiff bases compared to the Cs, characterizing different NMR signals according to the literature (Silverstein, Bassler, & Morrill, 1991). Therefore, NMR spectroscopy confirmed the chemical structures of all (CsB-β-KS)a-e derivatives.

The ¹H-NMR spectra showed the presence of multiple signals in the range of δ = 7.00–8.00 ppm, which were attributed to the aromatic sulfone moieties. In addition, the signals between δ = 1.99–2.01 ppm were from the acetyl proton, and another signal in the range of δ = 3.11–3.29 ppm was from the (H-2) proton of the pyranose ring. Furthermore, the ¹³C-NMR spectra showed the presence of aromatic moiety signals in the region of the aromatic frequencies

![Figure 1. The synthetic routes to β-ketosulfone akylation of metallic aryl sulfinate with α-halo ketones.](image)

| Compound No. | R  |
|--------------|----|
| ketosulfone-a | Br |
| ketosulfone-b | CH₃|
| ketosulfone-c | H  |
| ketosulfone-d | Cl |
| ketosulfone-e | F  |
in the range of $\delta = 128.5$–$134.4$ ppm, which was in accordance with the proposed structures of the (CsB-β-KS)a-e derivatives.

### 3.2. Characterization techniques for (CsB-β-KS)a-e

Normal characterization techniques were utilized to characterize the synthesized CsB-β-KS) a-e derivatives, including FT-IR, FE-SEM, XRD, and TGA thermal analysis.

The Fourier transform infrared spectroscopy (FT-IR) spectra of the pure Cs polymer and its corresponding (CsB-β-KS)a-e derivatives as the selective examples are shown in Figure 3. The FT-IR spectra were tested over the range of 400–4000 Cm$^{-1}$. Figure 3(f) shows the FT-IR spectrum of pure Cs, which exhibited a peak at 3305 cm$^{-1}$ for the asymmetric stretching vibration of the OH group, while an OH bend appeared at 1550 cm$^{-1}$. The amide group appeared at 1657 cm$^{-1}$, and the C-O stretching at 1085 cm$^{-1}$ was observed in all the modified Cs derivatives. The carbonyl group of the amide was shifted down at 1630 cm$^{-1}$, which might have been caused by the weak interaction of the hydrogen of the polymer host molecule with the carbonyl group, lengthening their bonds by decreasing the energy of the system. The FT-IR spectra of the corresponding (CsB-β-KS)a-e derivatives as the selected examples are shown in Figure 3(a–e). The absorbencies at 2950–2980 cm$^{-1}$ exhibited the presence of C-H asymmetric stretching vibrations. Similarly, the peak at 1100–1400 cm$^{-1}$ was caused by the presence of $S = O$ cm$^{-1}$ (sulfone group), and the $S-O$ cm$^{-1}$ vibrations appeared from 700 to 1000 cm$^{-1}$, as shown in Figure 3. In the region, a 1000–1100 cm$^{-1}$ C-O stretching vibration was observed as shown in the inset of Figure 3. The entire peak showed an N-H stretch at approximately 3400 cm$^{-1}$, while a C-O stretch at 1085 cm$^{-1}$ appeared in the modified Cs from Figure 3. Similarly, the amide group N-C = O and N-H deformation peaks appeared at 1650 and 1555 cm$^{-1}$ respectively, as shown in Figure 3.

The X-ray diffraction (XRD) analysis data of pure Cs and its corresponding (CsB-β-KS)a-e derivatives as the selective examples are shown in Figure 4 over the 2$\theta$ range 2$\theta$ = 10–80$^\circ$. In all the XRD diffractograms, broad signals at approximately 2$\theta = 20^\circ$ were observed, which confirmed the presence of the polymer matrices and the absence of metallic constituents. The XRD
The diffractogram of pure Cs showed a typical broad amorphous peak or sometimes referred to as the “semi-crystalline state” in the range of $2\theta = 15$–$25^\circ$. The peak maximum at approximately $2\theta = 20^\circ$ indicated an amorphous pattern of Cs for the highly disordered crystal structure. The minor signal at $2\theta = 38$–$42^\circ$ with a peak maximum at approximately $2\theta = 40^\circ$ (Islam et al., 2011; Kumar, Dutta, & Dutta, 2009; Ogawa, Yui, & Okuyama, 2004) and minor reflections at the lower $2\theta$ value of approximately $2\theta = 11^\circ$ were also considered as typical diffraction peaks of Cs for amorphous character (Bangyekan, Aht-Ong, & Srikulkit, 2006; Muzzarelli, Francescangeli, Tosi, & Muzzarelli, 2004). The $(\text{CsB-}\beta\text{-KS})_{a-e}^3$ derivatives also showed similar broad signals with minor reflections that shifted to higher $2\theta$ values at $2\theta = 25^\circ$. These reflection peaks indicated a lower degree of crystallinity and proved the strong interactions of the organic components with the electron donating groups of the Cs host molecule (Kumar et al., 2009; Ogawa et al., 2004).

The FE-SEM images of the pure Cs host molecule and its corresponding $(\text{CSB-}\beta\text{-KS})_{a-e}$ derivatives are shown in Figure 5. The $p$-halo-$\beta$-ketosulfone interacted with the active amino group in the Cs
polymer and enhanced its morphology. Therefore, the pure Cs surface morphology changed upon attachment with the p-halo-β-ketosulfone derivatives of variable loading because of the interaction of these derivatives to the polar centers located on the Cs polymer chains. The pure Cs images showed an undefined sheet-like morphology with little microcavities at lower and higher magnifications (x = 1500 and 7500), as shown in (Figure 5(a,b)). Figure 5(c–l) shows the FE-SEM images for the (CSB-β-KS)a-e3 derivatives as the selective examples. The attached derivatives adjusted on the Cs surface by the strong chemical bonding of the amine linkage made p-halo-β-ketosulfone derivatives with a constant number of hydrophilic sites at the product surface. The (CSB-β-KS)a3 images showed highly ordered sheets compared to that of the pure Cs in both magnifications at x = 1500 and x = 3000 (Figure 5(c,d), respectively). In addition, the (CSB-β-KS)c3 images showed needle-like particles. The average diameters of these needles were 65–200 nm, as shown in Figure 5(g,h) (magnification x = 1500 and 7500). In Figure 5(i,j), the images for the (CSB-β-KS)d3 derivative showed thick rod-like shapes using the same magnifications. The porosity of the Cs host molecule increased that of (CSB-β-KS)b3 and (CSB-β-KS)e3, providing a porous structure. The porous and spongy particle morphology was observed in the (CSB-β-KS)b3 and (CSB-β-KS)e3 FI-SEM images with patchy punctures in both cases. The average diameters for the punctures for the (CSB-β-KS)b3 derivative were higher than that in the (CSB-β-KS)e3 derivative. The measured puncture sizes were
Figure 5. FE-SEM of Pure Cs polymer (a, b), (CsB-β-KS)$_3$ (c, d), (CsB-β-KS)$_3$ (e, f), (CsB-β-KS)$_3$ (g, h), (CsB-β-KS)$_3$ (i, j) and (CsB-β-KS)$_3$ (k, l).
1–3 μm and 0.5–0.25 μm for the (CSB-β-KS)b₃ and (CSB-β-KS)e₃ derivatives, respectively, as shown in Figure 5(e,f,k,l) (magnification x = 1500, 3000, and 7500).

The thermal behaviors of the pure Cs and its corresponding (CSB-β-KS)a-e₃ derivatives were investigated by thermogravimetric analysis (TGA) in an air atmosphere and a heating rate of 10°C min⁻¹, as shown in Figure 6(a,b) and as listed Table 1. Figure 6(a) shows the thermal behavior for the pure Cs, which was in agreement with that reported in the literature (Fan, Li, Wang, Qian, & Jia, 2016; Maciel, Yoshida, & Franco, 2015; Rahman, Muraleedaran, & Abdul Mujeeb, 2015; Shruthi, Roy, Sailaja, & Sengupta, 2016). Pure Cs is considered one of the most important polysaccharides that carries hydrophilic groups in its main chain, including hydroxyl and/or amino groups. Therefore, as stated in the literature, the degradation occurs in two steps. The first mentioned degradation transition step is from the release of entrapped solvents, humidity, or attached moisture that easily and slowly releases and evaporates by heating the Cs. This process is nearly completed at
approximately 120°C. The second degradation step does not start before 240°C, the typical thermal stability of pure Cs. In the second stage, weight loss is detected over the temperature range 250–490°C, which is attributed to the final decomposition of pure Cs (Martinez-Camacho et al., 2010; Sheela, Nayaka, Viswanatha, Basavanna, & Venkatesha, 2012). PDT$_{\text{max}}$ represents the maximum temperature at which decomposition occurs. The PDT$_{\text{max}}$ value for pure Cs is 280°C, which is detected from its DTG curve, as shown in Figure 6(a). The TG curves of the (CSB-β-KS)$_{a-e}$ derivatives were measured over the thermal screening in the temperature range of 25–950°C, as shown in Figure 6(b). The (CSB-β-KS)$_{a-e}$ derivatives showed three steps of degradation as compared to the pure Cs. The rate of decomposition in the first and second steps was faster than that observed in the third step. From the TG curves, all (CSB-β-KS)$_{a-e}$ derivatives showed comparable thermal behavior over similar conditions with a slight increase in the reactive solvent for the thermal treatment. This was attributed to the higher stability of the Cs polymer matrix (Ray, Pal, Anis, & Banthia, 2010). The second and third degradation steps referred to the main decomposition steps for the tested derivatives and showed a slight overlap. The degradation occurred over a temperature range of 176–269°C and 270–613°C for the second and third steps, respectively. The TG curves also confirmed that the (CSB-β-KS)$_{a-e}$ derivatives performed as catalysts that promoted and/or expedited the degradation as compared to that observed in the pure Cs. This was attributed to the (CSB-β-KS)$_{a-e}$ derivatives decrease of the Van der Waals force interactions between the Cs chains and hence increase of the chain packing (Park et al., 2006). Furthermore, the $T_{25}$ and $T_{50}$ values (Asiri, Hussein, Abu-Zied, & Hermas, 2015; Hussein, Abu-Zied, & Asiri, 2016) represented the temperatures for the variable % weight loss at 25% and 50% weight loss, respectively, as listed in Table 1. The $T_{25}$ and $T_{50}$ values of pure Cs (269°C and 299°C) were considerably higher than those of the (CSB-β-KS)$_{a-e}$ derivatives; therefore, Cs showed moderate thermal stability at these weight losses. (CSB-β-KS)$_{d}$ showed the best thermal stability among the synthesized products, excluding Cs. Table 1 also lists PDT$_{i}$ and PDT$_{f}$ the initial and final polymer decomposition temperatures, respectively (Aly & Hussein, 2015; Hussein, 2018). Pure Cs showed a higher thermal behavior at PDT$_{f}$ than that of the other derivatives. Conversely, the PDT$_{i}$ values for all the derivatives (100–115°C) were significantly higher than that of Cs (59°C). The ranking of the PDT$_{i}$ values was (CSB-β-KS)$_{a}$ > (CSB-β-KS)$_{b}$ > (CSB-β-KS)$_{c}$ ≈ (CSB-β-KS)$_{d}$ ≈ (CSB-β-KS)$_{e}$. The (CSB-β-KS)$_{d}$ derivative showed the best thermal stability at PDT$_{i}$, even compared to pure Cs.

### 3.3. Antimicrobial activity

The evaluations of the inhibition zone and inhibition zone diameter with the selected target microbial pathogens were used to examine the in vitro antimicrobial activities of the desired Cs derivatives (CSB-β-KS)$_{a-e}$. The in vitro antimicrobial activities were tested for the following target microbial pathogens: two Gram-positive bacteria [Streptococcus pneumoniae (RCMB010010) and Bacillus subtilis (RCMB 010067)], two Gram-negative bacteria [Pseudomonas aeruginosa (RCMB 010043) and Escherichia coli (RCMB 010052)], and two fungi [Aspergillus fumigatus (RCMB 02568)]

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**Table 1. Thermal behavior of pure Cs and its corresponding (CSB-β-KS)$_{a-e}$ derivatives**

| Products          | PDT$_{i}$ (°C) | PDT$_{f}$ (°C) | Temperature (°C) for various percentage decompositions |
|-------------------|----------------|----------------|-------------------------------------------------------|
| Pure Cs           | 59             | 690            | 269, 299                                               |
| (CSB-β-KS)$_{a}$  | 115            | 622            | 199, 260                                              |
| (CSB-β-KS)$_{b}$  | 111            | 613            | 200, 263                                              |
| (CSB-β-KS)$_{c}$  | 102            | 612            | 195, 252                                              |
| (CSB-β-KS)$_{d}$  | 102            | 599            | 201, 265                                              |
| (CSB-β-KS)$_{e}$  | 100            | 611            | 199, 249                                              |

*The thermal behavior was determined in the air over a heating rate of 10°C min$^{-1}$.**
and *Candida albicans* (RCMB 05036)]. The standard antibacterial drug ampicillin (which is active against a wide range of Gram-positive bacteria), gentamicin (which is active against a wide range of Gram-negative bacteria), and the antifungal drug amphotericin B (used to treat serious fungal or yeast infections) were used to compare the antimicrobial results obtained from the Cs derivatives (CsB-β-KS)a-e. DMSO was used as the control and did not produce an inhibition zone. The obtained results of the examined Cs derivatives (CsB-β-KS)a-e showed moderate to good antibacterial activity for most strains of the bacteria tested (Table 2). Among the tested compounds, three derivatives (CsB-β-KS)α3, (CsB-β-KS)δ2, and (CsB-β-KS)γ3 showed good antibacterial activities against the Gram-positive and Gram-negative bacteria. All other compounds exhibited week and moderate activities. Compounds (CsB-β-KS)α2,2a, (CsB-β-KS)β1-a, and (CsB-β-KS)γ1-4, displayed the lowest activity, whereas compounds (CsB-β-KS)β1,a-e and (CsB-β-KS)γ1-4, displayed moderate activity. The inhibitory activities of (CsB-β-KS)γ3 against *Bacillus subtilis* (RCMB 010067) were more than threefold that of ampicillin. In addition, the inhibitory activities of compound (CsB-β-KS)δ2 against *Streptococcus pneumoniae* (RCMB010010) and *Bacillus subtilis* (RCMB 010067) were approximately similar to that of ampicillin. Conversely, the inhibitory activities of compound (CsB-β-KS)γ3 against *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052) were approximately similar to that of gentamicin. The tested compounds showed moderate to poor antifungal activity towards *Aspergillus fumigatus* (RCMB 02568) and *Candida albicans* (RCMB 05036), respectively.

The MIC of all compounds against the different types of bacteria was also determined, and the results are listed in Table 3. The MIC is the minimum concentration of an antimicrobial agent that will inhibit in vitro-growth of the infectious bacteria. Most of the synthesized compounds exhibited an adequate MIC against the Gram-positive bacteria than the Gram-negative bacteria. Among the tested compounds, (CsB-β-KS)α3 and (CsB-β-KSa)α2,3 had good MIC values against the Gram-positive bacterial strains. The MICs of these three compounds were equal to or less than the MIC of standard antibiotics. (CsB-β-KS)α3 and (CsB-β-KS)α2,3 were efficient bactericidal agents against Gram-positive bacteria with MICs of 4, 2, and 1 µg/mL. As a comparison, the antibiotic ampicillin has an MIC of 1.9 µg/mL. Moreover, most of the other compounds showed moderate to good antibacterial activity against the Gram-positive bacteria with MICs between 15 and 31.25 µg/mL, as listed in Table 3.

An analysis of the activity results indicated that the presence of a 4-chlorophenyl moiety in β-ketosulfone attached to the Cs with 15% loading affected the antibacterial activity. Therefore, the highest activity was observed for (CsB-β-KS)γ3 against the two different types of the Gram-positive bacteria used in this study.

### 3.4. Cytotoxic activity

The cellular toxicity of the modified Cs derivatives (CsB-β-KSa-e) was assessed in vitro by the Skehan et al. assay (Skehan et al., 1990). Multidose experiments on HCT, HEPG2, and MCF-7 were performed for (CsB-β-KS)α3, (CsB-β-KS)α2, and (CsB-β-KS)α3, which were the most active compounds against the test organisms. The three types of anticancer profiles against colon, liver, and breast cancer of the selected Cs derivatives are shown in Figures 7–9 respectively, and are listed in Table 4. The data generated was used to plot a dose-response curve to determine the concentration of the test compounds required to kill 50% of the cell population (IC50) (Figures 7–9). The results revealed that all the tested compounds showed variation in the inhibitory activity to the three different cancer cell lines in a concentration-dependent manner. The most effective compound on the HCT carcinoma cell line was (CsB-β-KS)α3, where increasing of the compound concentration decreased the cancer cells survival rate. The cancer cells recovered from the effect of (CsB-β-KS)α2. The most effective compound on the HEPG2 carcinoma cell line was (CsB-β-KS)α3. The (CsB-β-KS)α2 derivative showed no effect on the cancer cells. Conversely, all selected compounds had significant inhibitory effects on the MCF7 carcinoma cell line.

Furthermore, while comparing these three compounds, the results in Table 4 showed that (CsB-β-KS)α3 was the most effective compound in all cases. (CsB-β-KS)α3 inhibited all three types of cancer cells,
| Compounds | Gram-positive Bacteria | Gram-negative Bacteria | Fungi |
|-----------|------------------------|------------------------|-------|
|           | Streptococcus pneumoniae (RCMB 010010) | Bacillus subtilis (RCMB 010067) | Pseudomonas aeruginosa (RCMB 010043) | Escherichia coli (RCMB 010052) | Aspergillus fumigatus (RCMB 02568) | Candida albicans (RCMB 05036) |
| (CsB-β-KS)\text{a1} | 9.6 ± 0.4 | 12.5 ± 0.5 | NA | 12.9 ± 0.6 | NA | NA |
| (CsB-β-KS)\text{a2} | 10.5 ± 0.4 | 13.1 ± 0.3 | 8.3 ± 0.2 | 10.7 ± 0.3 | NA | NA |
| (CsB-β-KS)\text{a3} | 10.9 ± 0.47 | 117 ± 0.4 | 7.9 ± 0.3 | 9.8 ± 0.2 | NA | 8.5 ± 0.32 |
| (CsB-β-KS)\text{a4} | 10.9 ± 0.47 | 117 ± 0.4 | 7.9 ± 0.3 | 9.8 ± 0.2 | NA | 8.5 ± 0.32 |
| (CsB-β-KS)\text{b1} | 14.6 ± 0.58 | 14.3 ± 0.58 | 9.6 ± 0.4 | 12.5 ± 0.34 | NA | NA |
| (CsB-β-KS)\text{b2} | 12.6 ± 0.52 | 13.7 ± 0.58 | NA | 14.1 ± 0.37 | 9.1 ± 0.4 | 9.7 ± 0.4 |
| (CsB-β-KS)\text{b3} | 16.9 ± 0.42 | 17.6 ± 0.31 | 11.1 ± 0.42 | 11.4 ± 0.37 | NA | NA |
| (CsB-β-KS)\text{b4} | 14.6 ± 0.42 | 15.9 ± 0.53 | NA | 11.6 ± 0.42 | NA | NA |
| (CsB-β-KS)\text{c1} | 16.7 ± 0.36 | 19.2 ± 0.27 | 10.1 ± 0.32 | 10.9 ± 0.43 | 7.8 ± 0.3 | 10.1 ± 0.5 |
| (CsB-β-KS)\text{c2} | 18.3 ± 0.25 | 22.6 ± 0.44 | 134 ± 0.34 | 12.3 ± 0.47 | 11.6 ± 0.36 | 10.9 ± 0.21 |
| (CsB-β-KS)\text{c3} | 14.7 ± 0.06 | 16.1 ± 0.4 | 11.7 ± 0.41 | 10.8 ± 0.32 | 9.8 ± 0.34 | NA |
| (CsB-β-KS)\text{c4} | 10.8 ± 0.4 | 16.2 ± 0.8 | 12.1 ± 0.43 | 10.4 ± 0.24 | 11.3 ± 0.39 | 11.1 ± 0.42 |
| (CsB-β-KS)\text{d1} | 12.6 ± 0.25 | 19.0 ± 0.58 | 10.7 ± 0.34 | 11.9 ± 0.63 | 8.5 ± 0.4 | 10.8 ± 0.46 |
| (CsB-β-KS)\text{d2} | 19.7 ± 0.2 | 28.7 ± 0.2 | 11.8 ± 0.36 | 10.8 ± 0.44 | 11.6 ± 0.5 | 12.7 ± 0.37 |
| (CsB-β-KS)\text{d3} | 11.8 ± 0.31 | 13.7 ± 0.34 | 13.7 ± 0.51 | 18.9 ± 0.25 | NA | 10.5 ± 0.4 |
| (CsB-β-KS)\text{d4} | 11.2 ± 0.33 | 17.3 ± 0.44 | 10.1 ± 0.39 | 8.5 ± 0.37 | 10.7 ± 0.24 | 9.9 ± 0.4 |
| (CsB-β-KS)\text{e1} | 13.4 ± 0.58 | 19.6 ± 0.19 | 10.2 ± 0.32 | 9.4 ± 0.44 | NA | NA |

(Continued)
| Compounds | Gram-positive Bacteria | Gram-negative Bacteria | Fungi          |
|-----------|------------------------|------------------------|---------------|
|           | *Streptococcus pneumoniae* (RCMB 010010) | *Bacillus subtilis* (RCMB 010067) | *Pseudomonas aeruginosa* (RCMB 010043) | *Escherichia coli* (RCMB 010052) | *Aspergillus fumigatus* (RCMB 02568) | *Candida albicans* (RCMB 05036) |
| (CsB-β-KS)$_{e2}$ | 16.7 ± 0.3 | 22.4 ± 0.36 | 9.8 ± 0.27 | 11.9 ± 0.63 | 10.9 ± 0.4 | 9.2 ± 0.3 |
| (CsB-β-KS)$_{e3}$ | 14.3 ± 0.1 | 19.9 ± 0.3 | NA | 13.7 ± 0.4 | NA | 12.8 ± 0.4 |
| (CsB-β-KS)$_{e4}$ | 13.1 ± 0.3 | 16.3 ± 0.5 | 8.3 ± 0.1 | 13.3 ± 0.5 | 10.1 ± 0.3 | 13.2 ± 0.7 |
| Ampicillin | 23.8 ± 0.1 | 32.4 ± 0.3 | | | | |
| Gentamicin | | | 17.3 ± 0.1 | 19.9 ± 0.3 | | |
| Amphotericin B | | | | | 23.7 ± 0.1 | 25.4 ± 0.1 |

Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of clinically pathogenic microorganisms using (5mg/ml) concentration of tested samples. RCMB: Regional Center for Mycology and Biotechnology Culture Collection, NA: No activity.
Table 3. Minimum inhibitory concentration (MIC) of (CsB-β-KS)a-e against pathological strains based on serial dilution technique

| Compounds          | MIC     | Streptococcus pneumoniae (RCMB010010) | Bacillus subtilis (RCMB 010067) | Pseudomonas aeruginosa (RCMB 010043) | Escherichia coli (RCMB 010052) |
|--------------------|---------|--------------------------------------|---------------------------------|---------------------------------------|---------------------------------|
| (CsB-β-KS)a1       | 500     | 250                                  | >5000                           | 125                                   |
| (CsB-β-KS)a2       | 250     | 125                                  | 500                             | 125                                   |
| (CsB-β-KS)a3       | 2       | 2                                    | 1000                            | 250                                   |
| (CsB-β-KS)a4       | 125     | 250                                  | >5000                           | 250                                   |
| (CsB-β-KS)a5       | 15      | 15                                   | >5000                           | 250                                   |
| (CsB-β-KS)a6       | 125     | 125                                  | >5000                           | 62.5                                  |
| (CsB-β-KS)a7       | 125     | 62.5                                 | 500                             | 250                                   |
| (CsB-β-KS)b1       | 250     | 250                                  | >5000                           | 250                                   |
| (CsB-β-KS)b2       | 31.25   | 15.6                                 | 250                             | 250                                   |
| (CsB-β-KS)b3       | 125     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)b4       | 500     | 250                                  | 500                             | 250                                   |
| (CsB-β-KS)b5       | 125     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)c1       | 125     | 62.5                                 | 500                             | 250                                   |
| (CsB-β-KS)c2       | 250     | 62.5                                 | 500                             | 250                                   |
| (CsB-β-KS)c3       | 31.25   | 15.6                                 | 250                             | 250                                   |
| (CsB-β-KS)c4       | 125     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)c5       | 500     | 250                                  | 500                             | 250                                   |
| (CsB-β-KS)c6       | 125     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)c7       | 250     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)d1       | 250     | 62.5                                 | 500                             | 250                                   |
| (CsB-β-KS)d2       | 31.25   | 15.6                                 | 250                             | 250                                   |
| (CsB-β-KS)d3       | 125     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)d4       | 500     | 250                                  | 500                             | 250                                   |
| (CsB-β-KS)d5       | 125     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)d6       | 250     | 125                                  | 500                             | 250                                   |
| (CsB-β-KS)e1       | 125     | 62.5                                 | 500                             | 250                                   |
| (CsB-β-KS)e2       | 62.5    | 31.25                                | 500                             | 250                                   |
| (CsB-β-KS)e3       | 125     | 62.5                                 | >5000                           | 250                                   |
| (CsB-β-KS)e4       | 250     | 125                                  | 500                             | 250                                   |
| Ampicillin         | 1.9     | 0.98                                 | 31.25                           | 3.9                                   |
| Gentamicin         | 3.9     |                                      | 31.25                           |                                       |

MIC value (µg/ml) of the tested sample against tested microorganisms.
RCMB: Regional Center for Mycology and Biotechnology Culture Collection, NA: No activity

Figure 7. Inhibitory effects of (CsB-β-KS)a3, (CsB-β-KS)a2,3 derivatives on colon carcinoma cell line in vitro.
and its IC\textsubscript{50} values were 11.6 µg/mL for HCT, 15.8 µg/mL for HEPG2, and 11.2 µg/mL for MCF7. Although (CsB-β-KS)\textsubscript{a3} provided the highest IC\textsubscript{50} values against HEPG2 and MCF7 (23 and 22.9, µg/mL, respectively), a negative effect was shown against HCT. The ranking of the effective derivatives was as follows: (CsB-β-KS)\textsubscript{a3} > (CsB-β-KS)\textsubscript{d3} > (CsB-β-KS)\textsubscript{d2}, as listed in Table 4. In addition to, both compounds (CsB-β-KS)\textsubscript{a3} and (CsB-β-KS)\textsubscript{d2} exhibited low cytotoxic activity against the V79 normal cell line. (CsB-β-KS)\textsubscript{a3} exhibited no cytotoxic activity against the V79 cell line (Table 4). Noteworthy, DMSO did not seem to have any noticeable effect on cellular growth which used as negative control and Doxorubicin, a cancer chemotherapeutic drug, served as positive control.
Table 4. Anticancer activity of (CsB-β-KS)a-e (CsB-β-KS)d2 and (CsB-β-KS) d3 (IC50) against both three carcinoma cell lines and normal cell line V79

| Compound                  | IC50 (µg/ml)* |
|---------------------------|--------------|
|                           | HCT          | HEPG2        | MCF7         | V79          |
| (CsB-β-KS)d3              | 11.6         | 15.8         | 11.2         | >300         |
| (CsB-β-KS)d2              | ND           | ND           | 15.2         | >300         |
| (CsB-β-KS)d3              | ND           | 23           | 22.9         | ND           |
| Doxorubicin               | 2.07         | 3.16         | 1.79         | 3.4          |

*DMSO used as negative control and did not seem to have any noticeable effect on cellular growth.

4. Conclusion
A new category of Cs-based β-ketosulfone derivatives (CsB-β-KS)a-e was synthesized with good yield by the interaction of pure Cs with freshly prepared p-halo-β-ketosulfone derivatives in variable loading percentages (5%, 10%, 15%, and 20%) in a mildly acidic aqueous solution. The (CsB-β-KS)a-e derivatives were also characterized by various techniques, such as FT-IR, 1H-NMR, XRD, FE-SEM, and thermal analyses. The pure Cs surface morphology showed a noticeable change upon attachment with the p-halo-β-ketosulfone derivative. The (CsB-β-KS)a3 SEM images showed highly ordered sheets, while the (CsB-β-KS)b2 and (CsB-β-KS)e3 derivatives provided a porous structure. (CsB-β-KS)c3 showed needle-like particles. The average diameters of these needles were 65-200 nm. The (CsB-β-KS)d3 derivative showed a high thermal stability behavior at PDT. Pure Cs showed higher values than that of all derivatives at T25, T50, and PDT. A detailed biological screening of these derivatives was carried out against Gram-positive and Gram-negative bacteria and fungi. In addition, the synthesized products were evaluated as anti-cancer agents against three types of cancer cell lines, including HCT, HEPG2, and MCF-7. The (CsB-β-KS)a3 derivative was considered the most effective compound against all tested types of cancer cells, and its IC50 values were 11.6 µg/ml for HCT, 15.8 µg/ml for HEPG2, and 11.2 µg/ml for MCF7. The (CsB-β-KS)d3 derivative showed the highest IC50 values, 23 and 22.9, against HEPG2 and MCF7, respectively. Noteworthy, the modified Cs derivatives exhibited low cytotoxic activity or no cytotoxicity on normal cell line V79 which give these derivatives a great advantage for cancer drug discovery research.
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