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Research paper

# 2-((Benzimidazol-2-yl)thio)-1-arylethan-1-ones: Synthesis, crystal study and cancer stem cells CD133 targeting potential

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ABSTRACT

In order to develop a potent anti-tumor agent that can target both cancer stem cells and the bulk of tumor cells, a series of 2-((benzimidazol-2-yl)thio)-1-arylethan-1-ones **5a-o** was synthesized. All compounds were evaluated for their anti-proliferative activity towards colon HT-29 cancer cell line. In addition, their inhibitory effect against cell surface expression of CD133, a potent cancer stem cells (CSCs) marker, in the same cells was evaluated by flow cytometry at 10 μM. Compound **5l** emerged as the most active anti-proliferative analog against HT-29 ($IC_{50}$ = 18.83 ± 1.37 μM), that almost equipotent as 5-fluorouracil ($IC_{50}$ = 15.83 ± 1.63 μM) with 50.11 ± 4.05% inhibition effect on CD133 expression, suggested dual targeted effect. Also, compounds **5h, 5j, 5k** and **5m-o** inhibited the expression of CD133 with more than 50%. The SAR study pointed out the significance of substitution of the pendent phenyl group with lipophilic electron-donating groups or replacing it by 2-thienyl or 2-furyl groups.



## 1. Introduction

Cancer stem cells (CSCs) are a specific subpopulation of tumor cells that selectively possess tumor initiation and self-renewal capacity, as well as, the ability to give rise to bulk populations of non-tumorigenic cancer cell progeny through differentiation [1,2]. These cancer stem cells show bidirectional interactions with the cellular constituents of their individual niches, which enfold distinct developmental signaling networks, soluble mediators, and/or cell matrix processes. These interactions are essential for the establishment of a stem cell–permissive microenvironment and provide a crucial regulatory balance between self-renewal and differentiation and between quiescence and proliferation [3]. To be resilient to electromagnetic and chemical insults, to colonize other parts of the body and to be able to slumber for prolonged periods of time, CSCs are tacitly thought to have acquired the molecular armaments of normal stem cells: CSCs can renew themselves, and they are built to last a lifetime [4]. So, an intrinsic resistance to a variety of traditional chemo- or radiotherapies has been displayed by the CSCs [5,6], which elucidates the failure of many cancer therapies that kill the bulk of tumor cells but fail to eliminate CSCs which survive to regenerate new tumors. There are numerous studies which have identified cancer stem cells in leukemia [7], breast [8], brain [9], lung [10], colon [11], and others. CSCs in solid tumors are identified using an extensive list of markers, to name just a few, CD44, CD24, CXCR4, CDCP1 and CD133 [12]. CD133, also known as prominin-1, represents one of the most popular CSC markers. CD133 is a member of the prominin family of proteins and is a surface protein with five transmembrane domains initially discovered as a marker of hematopoietic stem cells [13] and of bone marrow-derived circulating endothelial progenitors involved in postnatal angiogenesis, inflammation and tissue regeneration [14,15]. Although being a reliable marker of stem/progenitor cells in normal adult tissues, such as the brain [16], liver [17], kidney [18] and prostate

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[19], CD133 antigen has been used as a CSC marker in various cancers, including those of the brain [20], pancreas [21], lung [22], ovarian [23], prostate [24] and colon [12].

According to the World Health Organization statistics, colon cancer is one of the leading causes of cancer related deaths worldwide and is more common in developed countries. Both drug resistance to chemotherapy and metastases are the major concerns in colon cancer therapy [25–27]. Regarding the colon cancer, the disease aggressiveness and evolution [28–30], treatment resistance [31–33], and survival [28] in colorectal cancer were found to be associated with high expression of CD133. Also, the prognostic value of circulating CD133 mRNA levels in colorectal cancer has been also reported [33–35]. All these findings suggest a crucial role of CD133 expressing cells in the initiation and evolution of human colon tumorigenesis and support its role as important CSC marker. Noteworthy, Many treatment protocols have been applied to colorectal cancer, but they have not resulted in a complete cure, most probably, due to the resistant colon CSCs. Therefore, it is important to develop therapeutics that target bulk tumor cells and CSCs simultaneously, in order to eliminate all cancer cells.

Among the heterocycles, benzimidazole has emerged as a promising class that possesses interesting biological activities including anticancer activity [36–40].

In view of the facts mentioned above and as part of our ongoing effort towards developing effective anticancer agents [41–43], herein we report the synthesis of certain 2-((benzimidazol-2-yl) thio)-1-arylethan-1-ones and biological evaluation of their antiproliferative activity towards colon HT-29 cancer cell line, in addition to, their inhibitory activity against cell surface expression of CD133 in HT-29 cancer cells by flow cytometry.

## 2. Results and discussion

### 2.1. Chemistry

Although A.A.O. Sarhan et al. [44] reported the reaction of 2-mercaptobenzimidazole **2** with aromatic ketones in acidified acetic acid, they did not separate the salts product, and directly neutralized the reaction mixture to obtain 2-benzimidazolylthioacetophenone derivatives. With the aim to separate the salt product in a good yield and to determine its definite structure, we modified the reported procedure [44]. While A.A.O. Sarhan et al. used few drops of concentrated $H_2SO_4$, we increased the used amount of concentrated $H_2SO_4$ up to two equivalents to obtain a quantitative yield of the salt after a short duration, about 30 min. X-ray crystallographic analysis for a salt product unequivocally revealed that its structure is 2-((2-oxo-2-phenylethyl)thio)-1*H*-benzo[*d*]imidazol-3-ium sulfate salt (Fig. 1). Subsequent neutralization of the prepared sulfate salts **4a-o** afforded the target 2-((benzimidazol-2-yl)thio)-1-arylethan-1-ones **5a-o** in an excellent yield 85–94% (Scheme 1).

Another synthetic strategy was utilized to prepare the target compounds **5a-o**. Bromination of aromatic ketones **3a-o** with copper (II) bromide in a refluxing mixture of chloroform and ethyl acetate afforded the corresponding phenacyl bromides **7a-o**. Then the reaction of **7a-o** with the potassium salt of 2-mercaptobenzimidazole **6**, furnished the target derivatives **5a-o** in a moderate yield 69–81% (Scheme 1).

All the prepared compounds were confirmed by spectral analyses which were in full agreement with the proposed structures.

### 2.2. X-ray crystallographic analysis

Crystals of compounds **4b** and **5n** were obtained by slow evaporation from solutions of ethanol (Figs. 1 and 2). The measurements of the crystals were performed on a Bruker SMART APEX II D8 Venture diffractometer with graphite-monochromated Mo *Kα* radiation (λ = 0.71073 Å) at 150 K. The structures were solved by direct method and refined with SHELXTL [45]. E-maps provided the positions of all the non-H-atoms. The full-matrix least-squares refinement was carried out on $F^2$'s using anisotropic temperature factors for all non-*H*-atoms. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Center and allocated with the deposition numbers: CCDC 1048412–1048413 and 1047318–1047319 for compounds **4b** and **5n**, respectively. The crystallographic data and

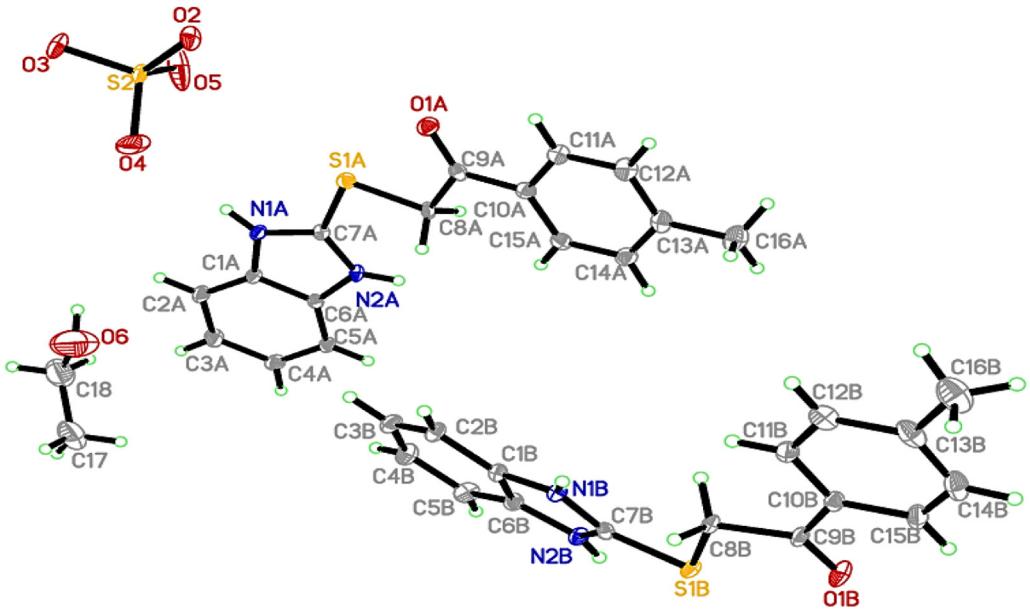


**Fig. 1.** An ORTEP diagram of final X-ray structure of compound **4b**.