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4-Bromophenylhydrazinyl benzenesulfonylphenylureas as indoleamine 2,3-dioxygenase inhibitors with *in vivo* target inhibition and anti-tumor efficacy

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ABSTRACT

Indoleamine 2,3-dioxygenase is a heme-containing enzyme implicated in the down regulation of the anti-tumor immune response, and considered a promising anti-cancer drug target. Several pharmaceutical companies, including Pfizer, Merck, and Bristol-Myers Squibb, are known to be in pursuit of IDO inhibitors, and Incyte recently reported good results in the phase II clinical trial of the IDO inhibitor Epacadostat. In previous work, we developed a series of IDO inhibitors based on a sulfonylhydrazide core structure, and explored how they could serve as potent IDO inhibitors with good drug profiles. Herein, we disclose the development of the 4-bromophenylhydrazinyl benzenesulfonylphenylurea **5k**, a potent IDO inhibitor which demonstrated 25% tumor growth inhibition in a murine CT26 syngeneic model on day 18 with 100 mg/kg oral administration twice daily, and a 30% reduction in tumor weight. Pharmacodynamic testing of **5k** found it to cause a 25% and 21% reduction in kyn/trp ratio at the plasma and tumor, respectively. In the CT26 tumor model, **5k** was found to slightly increase the percentage of $CD3^+$ T cells and lymphocyte responsiveness, indicating that **5k** may have potential in modulating anti-tumor immunity. These data suggest **5k** to be worthy of further investigation in the development of anti-tumor drugs.

1. Introduction

Cancer is a leading cause of death, accounting for 8.2 million deaths in 2012 [1]. Annual cancer cases are estimated to increase to 22 million over the next two decades [1], increasing demand for anti-cancer drugs. Recently, immunotherapy has emerged as a promising new strategy for cancer treatment. For example, Ipilimumab, a monoclonal antibody that blocks cytotoxic T lymphocyte antigen 4 (CTLA-4), was launched in 2011 for treatment of patients with metastatic melanoma. Nivolumab and Pembrolizumab, both launched in 2014, target programmed death-1 (PD-1) [2]. Atezolizumab and avelumab, launched in 2016 and 2017 respectively, target PD-L1. All of these biologics downregulate the anti-tumor immune response in the tumor microenvironment [3,4].

In addition to these well-studied immune-oncology targets (CTLA-4, PD-1, and PD-L1), indoleamine-2,3-dioxygenase (IDO) is also implicated in the down regulation of the anti-tumor immune response in the tumor microenvironment. The over-expression of IDO has been found in several cancer types, e.g., colorectal cancer, pancreatic cancer, non-small cell lung cancer, and glioblastoma; and was correlated with both tumor progression [5] and poor clinical outcome [6]. IDO was overexpressed in tumor cells upon exposure to pro-inflammatory cytokines, such as interferon-γ (INF-γ), that are produced by stimulated lymphocytes [7].

IDO activation leads to a tryptophan deficit, which induces the downregulation of activating natural killer (NK) cell receptor on NK

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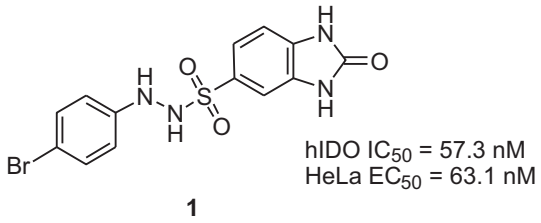


Fig. 1. Previously reported *N′*-(4-Bromophenyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-sulfonylhydrazide(**1**) as an IDO inhibitor.

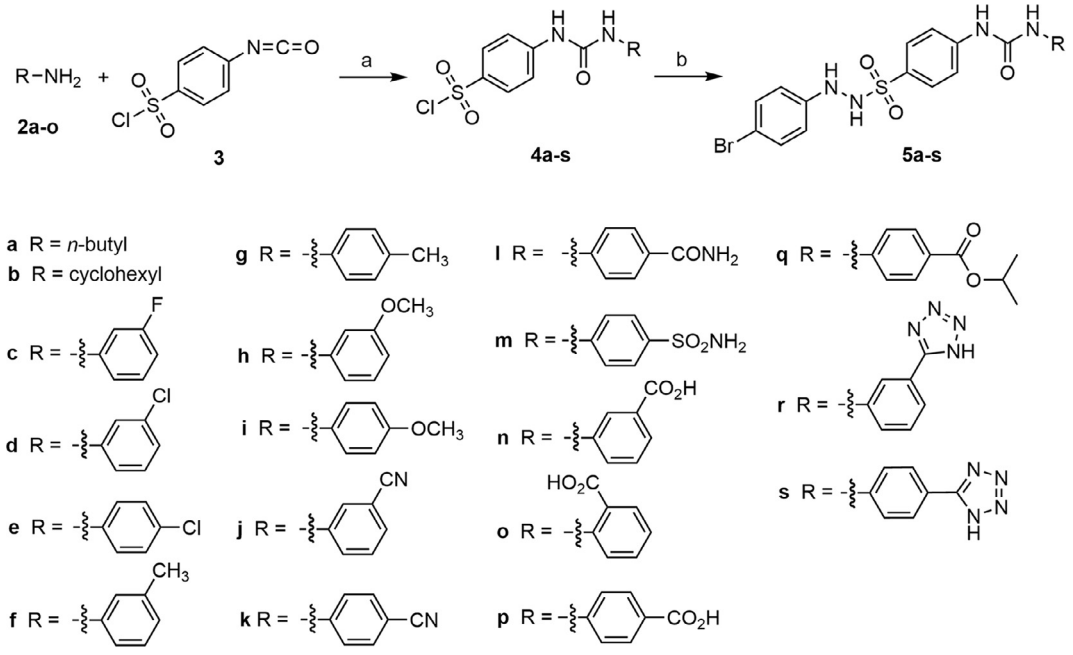
cells and inhibition of cytotoxic T cells by promotion of cell cycle arrest and apoptosis in the tumor microenvironment [8–10]. IDO$^+$-DCs modulate immune responses by the induction of lymphocyte cell cycle arrest, downregulation of the T cell receptor (TCR)ξ-chain, and induction of apoptosis, thereby preventing clonal expansion of antigen-specific lymphocytes [11–14]. On engagement with IDO$^+$DCs, lymphocytes become unreactive, and naive CD4$^+$ T cells are driven towards conversion into regulatory T (Treg) cells [5,6,11,15–18]. These IDO-positive DCs also inhibit activation of T cells by neighboring DCs that do not express IDO – a phenomenon called 'bystander suppression' [19,20]. IDO-positive DCs also inhibit natural killer T (NKT) cells and B cells. Invariant NKT cells change their cytokine secretion profile to a type 2 T helper cell pattern, and plasma cells decrease antibody production [14,21,22]. Thus, upregulation of IDO in the tumor microenvironment causes the tumor to escape immune surveillance, allowing its further development; whereas restriction of IDO activity leads to suppression of tumor progression, and restoration of anti-tumor immunity [6,17,23–26]. These findings establish IDO as an important molecular target for anticancer immunotherapeutics.

Epacadostat, developed by Incyte Corporation [26–28], is the most advanced known IDO inhibitor, and is currently in phase 3 clinical trials. In a recent phase 2 clinical study, Epacadostat was found to stabilize the disease progression of myelodysplastic syndromes of 12/15 patients for up to 12 months [29]. In addition, various classes of IDO inhibitors are being developed by several biotech companies [30–50].

Ongoing clinical studies of IDO inhibitors have sought to determine their efficacy both as single agents, and when administered in combination with either traditional chemotherapies, or with other cancer immunotherapies [31]. Using high-throughput screening, we discovered *N′*-phenethylbenzenesulfonylhydrazide as a potential IDO inhibitor, and subsequent structure-activity relationship (SAR) studies on the phenyl benzenesulfonylhydrazide scaffold resulted in the discovery of several compounds with potent IDO inhibitory activity in the nanomolar range [51,52], including compound **1**. Our previous work revealed that bromo-substitution at the *para*-position of the phenylhydrazinyl moiety and 2-oxo-benzimidazolesubstituent at the benzenesulfonyl end resulted in potent enzymatic activity against IDO as well as cellular activity (see Fig. 1) [51]. However, although compound **1** had potent *in vitro* activity, it was inactive *in vivo*, having unfavorable drug exposure ($AUC_{0\text{-inf.}}$ = 594 ng/mL h), high plasma clearance (57.1 mL/min/kg), short half-life (0.8 h), and poor oral bioavailability (13%) [52]. Herein, we disclose further lead optimization of phenyl benzenesulfonylhydrazide scaffold, wherein the benzenesulfonyl moiety was substituted with a variety of open chain substituted ureas, to give a series of 4-bromophenylhydrazinylsulfonylpheny lureas; the SAR, pharmacodynamic, pharmacokinetic, and animal pharmacology studies of which are reported.

2. Chemistry

The synthesis of 4-bromophenylhydrazinylsulfonylphenylureas **5a–s** is depicted in Scheme 1. 4-(Chlorosulfonyl)phenyl isocyanate (**3**) was reacted with primary amines **2a,b** and anilines **2c–s** to afford the 4-chlorosulfonylphenyl urea **4a–s** [53], which underwent coupling with 4-bromophenylhydrazines **4a–s** to give the corresponding 4-bromophenylhydrazinylsulfonylphenylureas **5a–s** in low to moderate yield (6–70%) [51,52]. The enzymatic-based and HeLa cell-based IDO activities for compounds **5a–s** were evaluated according to the procedures reported by us previously [51,52], and the results are shown in Table 1.



Scheme 1. Synthesis of 4-bromophenylhydrazinyl benzenesulfonylphenylureas (Reagents and conditions: (a) CH_2Cl_2, −78 °C, 1–1.5 h for **4a**,**b**; THF or CH_3CN, rt, 4 h–17 h for **4c–s** (14–99%). (b) 4-bromophenyl hydrazine, CH_2Cl_2 or DMF, rt, 3–18 h (6–70%)).