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Discovery and structure-activity relationships of phenyl benzenesulfonylhydrazides as novel indoleamine-2,3-dioxygenase inhibitors

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Abstract

A novel class of phenyl benzenesulfonylhydrazides has been identified as potent inhibitors of indoleamine 2,3-dioxygenase (IDO), and their structure-activity relationship was explored. Coupling reactions between various benzenesulfonyl chlorides and phenylhydrazides were utilized to synthesize the sulfonylhydrazides bearing various substituents. Compound **3i** exhibited 61 nM of IC_{50} in enzymatic assay and 172 nM of EC_{50} in the HeLa cell. The computational study of **3i** suggested that the major interactions between **3i** and IDO protein are the coordination of sulfone and heme iron, the hydrogen bonding and hydrophobic interactions between **3i** and IDO. This novel class of IDO inhibitor provides a new direction to discover effective anti-cancer agents.

Keywords

Sulfonylhydrazide

Indoleamine 2,3-dioxygenase

Structure-activity relationship

Molecular docking

Cancer continues to be a leading cause of death around the world, and developing new therapeutics is urgently needed.[1] In addition to chemotherapeutic and targeted agents, cancer immunotherapy is an emerging approach in anti-cancer therapy. The recently approved ipilimumab along with sipuleucel-T has validated the principle, and both extend survival of cancer patients. Several approaches including immune-checkpoint modulators are intensively under exploration and development.[2-6] Indoleamine 2,3-dioxygenase (IDO) is also a molecular target under investigation.

Indoleamine 2,3 dioxygenase (IDO) is a heme-containing enzyme which degrades tryptophan in the kynurenine pathway. Depletion of tryptophan and production of bioactive metabolites upon IDO induction exerts T cells to become more sensitive to apoptosis and cell cycle arrest. In addition, T cells are prone to differentiate into immunosuppressive regulatory T cells under these conditions.[7–9] A mounting body of evidence indicates that overexpression of IDO is correlated with tumor progression and invasiveness, and its expression is associated with poor prognosis.[7,9] In pre-clinical models, blocking IDO activity results in tumor regression in tumor-bearing syngeneic mouse models.[10,11]

Several IDO inhibitors have been discovered. 1-methyl-DL-tryptophan (1-MT), with K_i ~ 34 μM, has been well studied, and its D form is now in clinical trials. Although it exhibits limited IDO inhibitory activity *in vitro*, satisfactory anti-tumor activity *in vivo* has been reported.[10] Natural product-derived IDO inhibitors such as brassinin,[12] MTH-trp,[13] naphthaquinone,[14] exiguamine A,[15] tryptanthrins,[16] and benzofuranquinones [17] have been reported too, and some of these exhibit potent IDO inhibitory activity *in vitro*. Non-natural product-derived IDO inhibitors include 4-phenylimidazole,[18] phenyl-triazoles,[19,20] indol-2-yl ethanones,[21] benzothiazole,[22] candesartan,[23] oxadiazolylcarboximidamides,[24] and *S*-benzylisothioureas,[25] some of which inhibited tumor growth *in vivo*. Recently, the small molecule IDO inhibitor INCB024360 was reported to be well-tolerated in patients and effective in highly refractory patients.[26] These results support the validity of IDO inhibition as a potential immunotherapeutic strategy for cancer treatment. In this study, a novel

structural scaffold of IDO inhibitors will be reported, its analogs synthesized, and its structure-activity relationships discussed.

A high-throughput screening assay was carried out on an in-house library by measuring kynurenine formation in an enzymatic assay with purified recombinant human IDO protein, [27] and 2-phenyl benzeneethanesulfonylhydrazide (**1a**) was identified as a potent IDO inhibitor (IC_{50} = 167 nM, figure 1). However, it did not show any cellular effect in inhibiting IDO activity. This result suggests that **1a** was not capable of entering into cells and modifications to improve cell permeability would be required. This promising hit was further modified and a series of phenyl benzenesulfonylhydrazides (**1b**–**1f**) were synthesized and assessed in the enzymatic assay (IC_{50}) and the Hela cell-based cellular assay (EC_{50}).

Insert Figure 1

Methods for synthesis of phenyl benzenesulfonylhydrazides have been well developed.[28–33] Coupling reactions of substituted phenyl hydrazines with commercially available sulfonyl chlorides were used to prepare a series of phenyl benzenesulfonylhydrazides. Initially, *N*'-(3-methylphenyl)benzenesulfonylhydrazides **1b–1f** were synthesized and their biological activities examined (Scheme 1). The resulting compounds **1b–1f** inhibited IDO enzymatic activity with IC_{50} values of less than 100 nM. Notably, compound **1b** displayed cellular IDO inhibitory activity in the HeLa cell line with an EC_{50} value of 241 nM.

Insert Scheme 1

Based on the data shown in scheme 1, the acetamido group at the *para*-position of the benzene ring of the benzenesulfonyl moiety resulted a molecule with good cellular activity (**1b**, EC_{50} = 241 nM). In order to obtain even more potent compounds, the position of the acetamido group of the benzenesulfonyl moiety was fixed, and various substituted phenyl hydrazines were introduced to

give the corresponding phenyl benzenesulfonylhydrazide derivatives. As shown in Table 1, compounds **2a–2t** were prepared by conditions similar to that for compounds **1b–1f**. Compound **2b** (IC_{50} = 51 nM), in which R^3 is a methyl group, inhibited IDO activity more potently than **1a**. *p*-Methoxy compound **2c** (IC_{50} = 254 nM) was slightly less active than **2b** against IDO. However, **2b** and **2c** showed poor or no cellular IDO inhibitory activity. Compounds **2d–2f,** in which R^3 is an electron-withdrawing group exhibited poor inhibitory activity against IDO (IC_{50} = 1605 nM–5667 nM).

Next, the effect of halogen substituent on the phenyl ring of the phenylhydrazine was examined. Compound **2g**, with a fluoride at R^3, was found to be a potent inhibitor of IDO in enzymatic assay (IC_{50} = 79 nM), with modest inhibition of IDO in the HeLa cell line (EC_{50} = 609 nM). 4-Bromo compound **2i** exhibited potent cellular activity (EC_{50} = 85 nM) with an IC_{50} value comparable to **2g** (IC_{50} = 130 nM). Introducing a halogen at R^2 resulted in a dramatic loss of IDO cellular potency, even though the enzymatic activities of **2j** and **2k** were maintained (IC_{50} = 122 and 255 nM, respectively). Addition of a *p*-fluoro substituent to these derivatives improved IDO cellular potency slightly, without significantly affecting the IC_{50} value (**2l**, IC_{50} = 128 nM, EC_{50} = 2494 nM; **2m**, IC_{50} = 110 nM, EC_{50} = 2588 nM). Di-substituted compounds such as 4-bromo-3-fluoro (**2n**), 4-bromo-3-methyl (**2o**) and 4-chloro-3-fluoro (**2p**) exhibited good IDO enzymatic and cellular potencies (IC_{50} = 50–194 nM; EC_{50} = 84–187 nM). A variety of other compounds bearing a di-substituted phenyl hydrazine ring (**2q–2t**) were less active than **2n–2p** in both enzymatic and cellular assays.

Insert Table 1

As shown in table 1, bromo-substitution at the 4-position of the phenylhydrazinyl moiety resulted in potent enzymatic activity as well as cellular activity (**2i**). To further investigate the role played by substituents on the benzenesulfonyl moiety, the synthesis of a number of derivatives

(**3a–3i**) was carried out. The chemical structures and biological activities of these compounds are shown in Table 2. Most were found to be potent IDO inhibitors with IC_{50} values of less than 180 nM, except **3c** (IC_{50} = 1431 nM). Compounds **3b** and **3i** had IC_{50} values of less than 100 nM.

Insert Table 2

To investigate the role of free NH-group at N_2-position of phenyl benzenesulfonylhydrazide, the N_2-methyl phenyl benzenzsulfonylhydrazide (**4**) was synthesized (figure 2). The inhibitory activity of compound **4** (IC_{50} = 4055 nM) was greatly inferior to that of compound **2i** (IC_{50} = 130 nM), suggesting that the NH-group at N_2-position of phenyl benzenesulfonylhydrazide greatly contributes to the inhibition of IDO.

Insert Figure 2

A computational study was performed to elucidate the interactions between the most potent compound, **3i**, and IDO (figure 3). Compound **3i** was docked into the inhibitor binding site of IDO by GOLD 5.1 using the structure of IDO in complex with the compound, 4-phenylimidazole (PDB code 2D0T), as the template. One of the oxygen atoms of the sulfone group of **3i** was found to coordinate to the heme iron with a distance of 2.04 Å. The other oxygen atom of the sulfone group formed a hydrogen bond with the main chain NH group of Ala264. The bound conformation of **3i**, which adopted a V-shape to bind to the protein, occupied both Pocket A and Pocket B. The benzoic acid moiety of **3i** formed a hydrogen bond with the thiol group of Cys129, and hydrophobic contacts with the surrounding residues in Pocket A, including Tyr126, Cys129, Phe164, Ser167,

Gly262, Ser263, and Ala264. The bromine group of phenyl hydrazine moiety extended into the hydrophobic cavity of Pocket B, formed by Phe163, Phe226, Arg231, Leu234, Ile354, and the heme ring.

Insert Figure 3

In conclusion, a novel series of phenyl benzenesulfonylhydrazides has been synthesized and identified as potent IDO inhibitors. A one step synthetic reaction of phenylhydrazines with sulfonyl chlorides was used to efficiently synthesize the desired phenyl benzenesulfonylhydrazides. Most of the analogs studied showed potent enzymatic IDO inhibitory activities. The computational study performed on compound **3i** suggests that the coordination formed between the oxygen of the sulfone group and the heme of IDO together with the hydrogen bond formed between the benzoic acid of **3i** and the thiol group of Cys129 are the major interactions between **3i** and the enzyme. In addition, the hydrophobic interaction between the bromide group and phenyl ring of **3i** with pocket A and B of IDO also contribute to the activity. Two of the phenyl benzenesulfonylhydrazides exhibited potent IDO inhibitory activities in the cell-based assay (**2i** and **2p**). Both halogen-containing phenylhydrazinyl and acetamido-substituted benzenesulfonyl moieties were found to be necessary for potent inhibitory activity in the cellular assay. This study presents a novel IDO inhibitor and its structure-activity relationships study is investigated. These results provide a new direction to discover potent IDO inhibitors for the treatment of cancer.

Acknowledgements

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Supplementary data

Identification data of chemical compounds and biological assay protocols are available in the online version.

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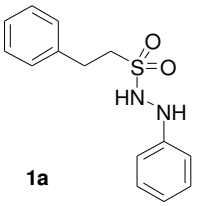
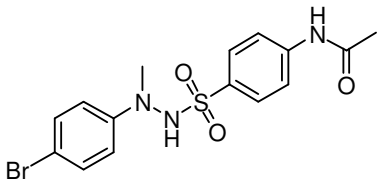


1a

hIDO IC_{50} = 167 nM
HeLa EC_{50} > 10 μM

Figure 1. High-throughput screening hit



hIDO IC_{50} = 4055 nM

Figure 2. Structure of compound **4**.

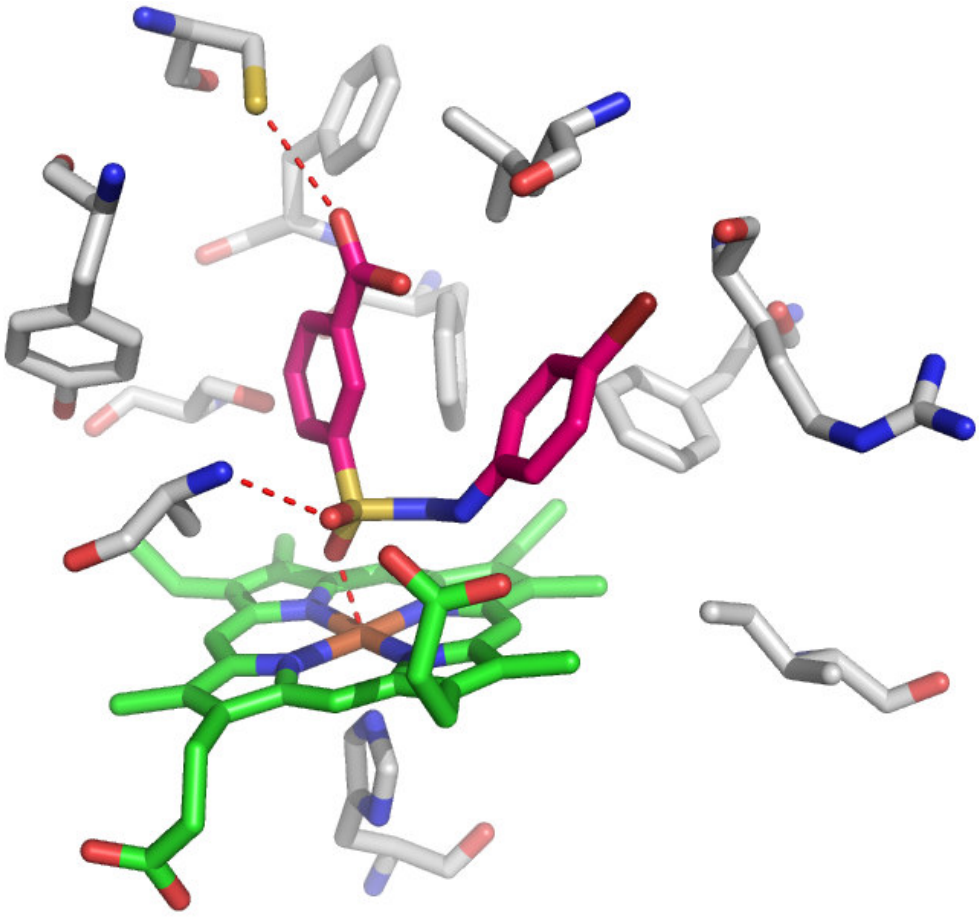
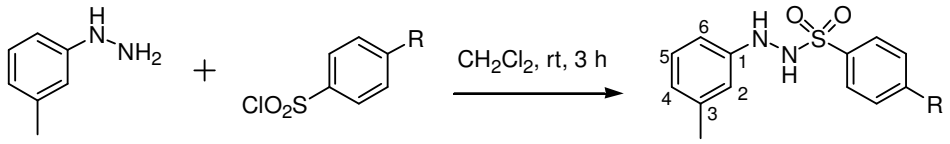
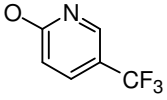


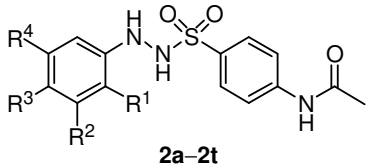
Figure 3. Computational binding mode of **3i** (magenta) at the IDO active site (PDB code 2D0T). Carbon atoms are shown in gray/green/magenta, nitrogen in blue, oxygen in red, bromine in crimson, sulfur in yellow, and iron in tan. Hydrogen atoms are omitted for clarity.



1b, R = NHAc (IC_{50} = 49±5 nM; EC_{50} = 241±52 nM)
1c, R = $CONHCH_2COOH$ (IC_{50} = 80±5 nM; EC_{50} = 571±65 nM)
1d, R = SO_2CH_3 (IC_{50} = 43±21 nM; EC_{50} = 7846±1029 nM)
1e, R = CN (IC_{50} = 59±2 nM; EC_{50} = 3404±607 nM)
1f, R =  (IC_{50} = 59±17 nM; EC_{50} = 296±59 nM)

Scheme 1. Synthesis and EC_{50} values of *N*'-(3-methylphenyl)benzenesulfonyl hydrazides **1b–1f**.

Table 1. Structures and IDO inhibitory activities of phenyl benzenesulfonylhydrazides **2a**–**2t**

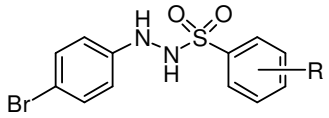


2a–**2t**

compounds	R^1	R^2	R^3	R^4	IC_{50} (nM)[a]	EC_{50} (nM)[a]
2a	H	H	H	H	71±7	>10000
2b	H	H	Me	H	51±6	1171±70
2c	H	H	OMe	H	254±22	>10000
2d	H	H	CN	H	5667±1374	1393±159
2e	H	H	SO_2NH_2	H	2475±524	3118±509
2f	H	H	CF_3	H	1605±196	181±17
2g	H	H	F	H	79±35	609±16
2h	H	H	Cl	H	155±24	142±12
2i	H	H	Br	H	130±27	85±4
2j	H	F	H	H	122±31	>10000
2k	H	Br	H	H	255±28	7563±1324
2l	H	Br	F	H	128±21	2494±71
2m	H	Cl	F	H	110±26	2588±136
2n	H	F	Br	H	205±1	187±7
2o	H	Me	Br	H	58±15	130±9
2p	H	F	Cl	H	194±38	84±15
2q	H	F	H	F	1746±15	2229±264
2r	F	H	F	H	608±105	>10000
2s	H	Cl	Cl	H	247±57	350±34
2t	H	Cl	H	Cl	352±70	508±77

[a] IC_{50} and EC_{50} values are the mean of at least three independent assays, presented as mean ± SD.

Table 2. Structures and IDO inhibitory activities of phenyl benzenesulfonylhydrazides **3a–3l**



3a–3l

compounds	R	IC_{50} (nM)[a]	EC_{50} (nM)[a]
3a	H	172±29	128±5
3b	4-Br	85±6	374±55
3c	4-OMe	1431±346	640±55
3d	4-CN	176±3	134±7
3e	4-$(CH_2)_2CO_2Me$	121±19	465±26
3f	4-$NHCO(CH_2)_2CH_3$	132±21	187±4
3g	4-$(CH_2)_2CO_2H$	141±36	183±7
3h	4-OCH_2CO_2H	125±8	152±6
3i	CO_2H	61±12	172±17

[a] IC_{50} and EC_{50} values are the mean of at least three independent assays, presented as mean ± SD.

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