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Synthesis of 4- and 5-arylthiazolinethiones as inhibitors of indoleamine 2,3-dioxygenase.

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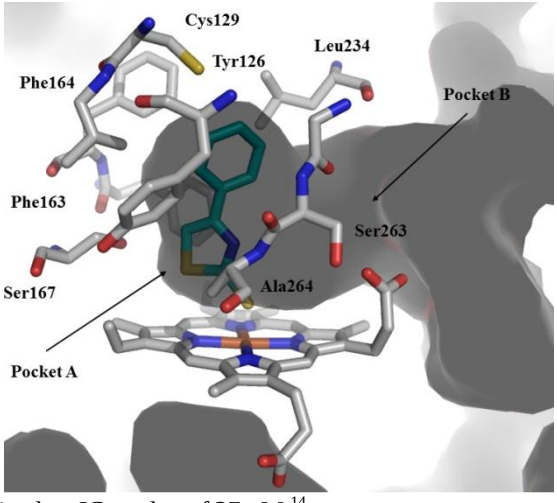
ABSTRACT

Docking studies of 4-phenylthiazolinethione on human IDO1 suggest complexation of the heme iron by the exocyclic sulfur atom further reinforced by hydrophobic interactions of the phenyl ring within pocket A of the enzyme. On this basis, chemical modifications were proposed to increase inhibition activity. Synthetic routes had to be adapted and optimized to yield the desired substituted 4- and 5-arylthiazolinethiones. Their biological evaluation shows that 5-aryl regioisomers are systematically less potent than the corresponding 4-aryl analogs. Substitution on the phenyl ring does not significantly increase inhibition potency, except for 4-Br and 4-Cl derivatives.

Human Indoleamine 2,3-DiOxygenase (IDO1, EC 1.13.11.52) is overexpressed constitutively in many human tumors[1] and is correlated with poor survival prognosis in several cancer types.[2,3] This protein is an extrahepatic heme-containing enzyme that catalyzes the catabolism of tryptophan (Trp) in the initial rate-limiting step of the kynurenine pathway. Consequent local depletion of Trp blocks proliferation of T lymphocytes resulting in cancer immune escape. *In vitro* and *in vivo* studies suggest that the efficacy of therapeutic vaccination or chemotherapy could be improved by concomitant administration of an IDO1 inhibitor, making IDO1 a new therapeutic target.[4,5]

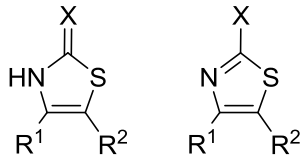
IDO1 degrades different indoleamine substrates including L-Trp, D-Trp, serotonin, and tryptamine, which makes this enzyme less selective than the related tryptophan 2,3-dioxygenase (TDO) which is specific for Trp. Experimental and theoretical evidence strongly supports a mechanism for IDO1 that involves binding of both the substrate and molecular oxygen to the distal heme site.[6,7] The enzyme catalyzes the cleavage of the pyrrole ring of the substrate and incorporates both oxygen atoms before releasing N-formylkynurenine, which is subsequently hydrolyzed to kynurenine by cytosolic formamidase. The first crystal structures of IDO1 included heme-bound cyanide or 4-phenylimidazole.[8] More recently, crystal structure of human IDO1 in complex with the thiazolotriazole compound Amg-1 (PDB: 4PK5) shows a shift of both Phe226 and Arg231 residues, located in pocket B.[9] Further investigations[10] have recently highlighted that interactions with those residues are essential for IDO1 inhibition.

As part of our interest for the design of original inhibitors of human IDO1,[11-12] we set up a medicinal chemistry program starting from 4-phenylthiazolinethione (**1**, Table 1) as a lead compound. Inhibition of human IDO1 by this molecule was first reported by Röhrig *et al.*[13] as part of an *in silico* screening approach. We confirmed the inhibitory activity of molecule **1** and



determined an IC_{50} value of 37 μM.[14]

Figure 1. Docking solution of compound **1** into the binding site of human IDO1. Docking was performed using Gold, starting from the crystal structure coordinates of the enzyme bound to 4-phenylimidazole (PDB entry 2D0T).



1 - 3

4 - 6

	X	R^1	R^2	%Inh @100 µM	% Inh @10 µM
1	S	C_6H_5	H	76±1	35±1
2	S	H	C_6H_5	16±4	-
3	O	C_6H_5	H	15±2	-
4	NH_2	C_6H_5	H	13±3	-
5	Cl	C_6H_5	H	0.5±2	-
6	Br	C_6H_5	H	12±2	-
1a	S	2-(C=O)OH-C_6H_4	H	8±2	-
1b	S	2-OCH_3-C_6H_4	H	53±0	-
1c	S	2-F-C_6H_4	H	77±1	-
1d	S	3-Br-C_6H_4	H	81±2	-
1e	S	3-CH_2OH-C_6H_4	H	16±1	-
1f	S	3-F-C_6H_4	H	28±1	-
1g	S	3-Cl-C_6H_4	H	40±1	-
1h	S	4-CH_3-C_6H_4	H	90±0	39±3
1i	S	4-OCH_3-C_6H_4	H	68±1	-
1j	S	4-CN-C_6H_4	H	8±2	-
1k	S	4-CF_3-C_6H_4	H	37±1	-
1l	S	4-F-C_6H_4	H	72±2	-
1m	S	4-Cl-C_6H_4	H	95±3	79±1
1n	S	4-Br-C_6H_4	H	100±2	88±1
1o	S	3-pyridine	H	48±2	-
1p	S	4-pyridine	H	21±4	-
2a	S	H	2-OCH_3-C_6H_4	12±0	-
2b	S	H	2-F-C_6H_4	29±2	-
2c	S	H	3-Br-C_6H_4	48±1	-
2d	S	H	4-CH_3-C_6H_4	15±4	-
2e	S	H	4-OCH_3-C_6H_4	0	-

Table 1 : Human indoleamine 2,3-dioxygenase inhibition (inhibition % at 100 µM) of substituted 4- and 5-arylthiazolinethiones synthesized as shown on .chemes 2, 3 and 4. Substrate (L-Trp) concentration was 100 µM, enzyme concentration 5µg/mL. The premix used to reduce iron from the heme is composed of ascorbic acid (10^{-2} M), methylene blue (5.10^{-5} M) in phosphate buffer (5.10^{-2} M, pH 6.5). Final DMSO concentration is 5%. Data are the mean of measurements made in triplicate

The nature of the heterocycle has been evaluated with compounds **1**-**6** (Table 1) and this analysis confirms that the thiazolinethione (X = S) is preferred. We have further confirmed by a combination of crystallography and *ab initio* calculations that the most stable tautomer of compound **1** is the thiazolinethione.[15]

The starting point of our study was the docking of compound **1** into the active site of human IDO1. The crystal structure coordinates of the enzyme bound to 4-phenylimidazole (PDB entry 2D0T) were used as input geometry for our structure-based approach. Docking solutions (Gold program,[16] Goldscore scoring function) suggest that 4-phenylthiazolinethione occupies pocket A of the distal heme site (Figure 1), the exocyclic sulfur atom of the molecule binding the iron cation of the heme cofactor and the phenyl ring fitting the binding pocket above the cofactor, similarly to what is observed in the complex with 4-phenylimidazole. The binding site (pocket A) consists of residues Tyr126, Cys129, Val130, Phe163, Phe164, Ser167, Leu234, Gly262, Ser263, Ala264, and the heme ring. This docking study suggests that only small substituents on the phenyl ring of **1** could be accommodated in pocket A. The influence of small substituents on the inhibition of human IDO1 was therefore evaluated by preparing a series of analogues of 4-phenylthiazolinethione (**1a-p**, Table 1).

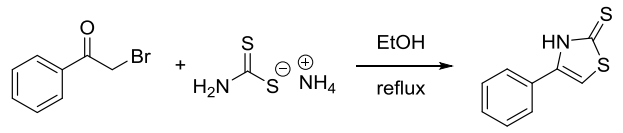
The analysis of the residues present in the binding site suggested the possibility of formation of a hydrogen bond with Cys129. Alternative possible hydrogen bonding sites are the hydroxy group of Ser167, the CO group of Gly262 backbone, and the NH group of Ala264 backbone or the 7-propionate group of the heme cofactor. An attempt to interact with these residues of the binding pocket, was made by preparing two isomeric pyridine derivatives of **1** (**1o** and **1p**).

The position of the phenyl ring on the thiazolinethione was also suggested to play a role on the inhibition by the molecules. This was tested by preparing several 5-arylthiazolinethiones (**2, 2a-e**, Table 1).

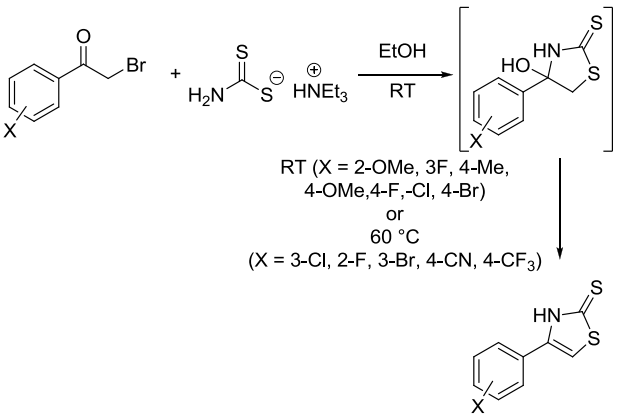
The preparation of the targeted molecules, in particular the 5-arylthiazolinethiones, needed the development of a new synthetic route. In order to obtain the 4-aryl derivatives, we turned to the classical synthesis of thiazolinethiones from α-bromoketones and ammonium dithiocarbamate (Scheme 1). However, ammonium dithiocarbamate is not commercially available. Even though its preparation and isolation are well described[17] it is known to be unstable towards air and decomposes upon storage. Furthermore, we quickly became aware of the limited solubility of ammonium dithiocarbamate, even in polar organic solvents. We nevertheless synthesized **1** by the classical method using ammonium dithiocarbamate in refluxing ethanol, with variable results in terms of reproducibility (Scheme 1). We also observed the formation of unidentified side-products that might arise from decomposition of ammonium dithiocarbamate.

In order to improve the solubility of the reagent, we prepared triethylammonium dithiocarbamate and were delighted to find that it reacts efficiently with the appropriately substituted alpha-bromoketones to afford the desired 4-aryl thiazolinethiones (**1, 1a-p**) with good yields (72-93%) within only 10 min. of reaction time (Scheme 2). Most reactions were performed at room

temperature while heating (60°C) was required for compounds **1c, 1d**, **1g**, **1j**, and **1k** to allow dehydration to the desired product.

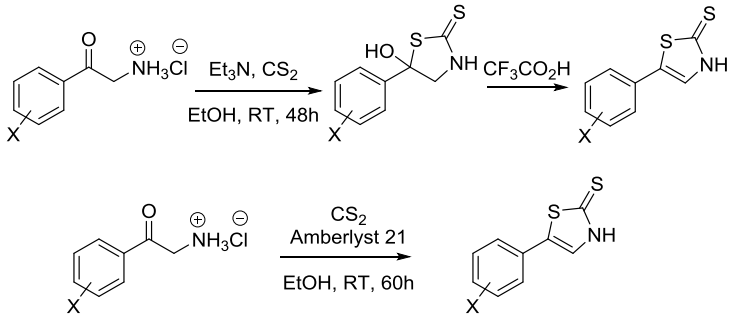


Scheme 1. Synthetic route to 4-phenylthiazolinethione **1**

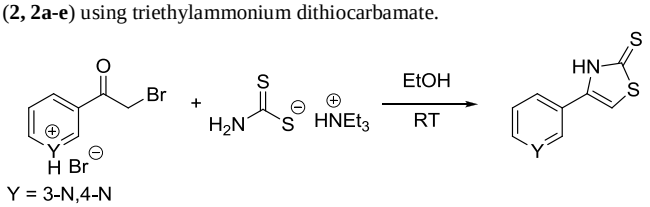


Scheme 2. Optimized synthetic route to desired substituted 4-arylthiazolinethiones (**1a-p**) using triethylammonium dithiocarbamate.

The synthesis of 5-arylthiazolinethiones was first attempted starting from the corresponding α-aminoketones using triethylamine and CS_2 in EtOH followed by dehydration of the 5-hydroxythiazolidine-2-thione intermediates with CF_3COOH.[19] Yields were good (68-76%) for long reaction times (48h) but purification of the products was tedious due to contamination by triethylamine. Replacement of triethylamine by a solid-supported base (Amberlyst 21) simplified the purification, although reaction times were longer. This optimized procedure (Scheme 3) avoids the acidification step and the final yields are generally higher (80%).



Scheme 3. Optimized synthetic routes to substituted 5-arylthiazolinethiones (**2, 2a-e**) using triethylammonium dithiocarbamate.



Scheme 4. Optimized synthetic route to pyridinylthiazolinethiones (**1o**, Y=4-N; **1p**, Y=3-N) using triethylammonium dithiocarbamate.

Pyridyl thiazolinethiones (**1o** and **1p**) were obtained by condensation of triethylammonium dithiocarbamate with appropriate pyridylbromo ketones in ethanol (Scheme 4). The starting 3- and 4-pyridyl-2-bromoethanone are readily prepared from the corresponding acetylpyridines using Br_2 in HBr/HOAc[20]. Compounds **1o** and **1p** precipitate after 2 hours of reflux of the reaction mixture and are isolated by filtration. Triethylamine is removed by trituration in ethanol leading to desired products with good purity and yield (81% and 87%).

The synthesized products were characterized by IR, ^{1}H NMR, ^{13}C NMR, and LCMS. Experimental details and characterizations are provided as supplementary material. Single crystals of compounds **1b** and **1h** were also obtained and unambiguously confirmed the assigned structure. In both cases, the thiazolinethione tautomers, and not the mercaptothiazole, were observed.

The biological activity of the synthesized 4- and 5-aryl thiazolinethiones **1a-p**, **2** and **2, 2a-e** was evaluated using a colorimetric *in vitro* IDO1 inhibition assay.[21,22] Briefly, the inhibitors are incubated (10 min, 37°C) with purified human IDO1 and L-Trp, the natural substrate. Trichloroacetic acid is then added to quench the reaction and the N-formylkynurenine generated is hydrolyzed to kynurenine during the next 30 min. *p*-Dimethyl-amino-benzaldehyde is added to the reaction in order to react with kynurenine and form a Schiff base. The absorbance of this compound is measured at 490 nm and is used to calculate the residual activity of IDO1. The resulting inhibition activities are presented in Table 1. For the most active molecules, the following IC_{50} values were determined: IC_{50} = 24 μM for **1,** 5.9 μM for **1h** (X =4-Me), 2.6 μM for **1m** (X =4-Cl), and 3.4 μM for **1n** (X =4-Br). These values are consistent with the inhibition percentages measured at 100 μM. Dose-response curves and additional details are provided as supplementary material.

From the IDO1 inhibition properties of the compounds under study (Table 1) a series of conclusions can be drawn:

- The nature of the heterocycle plays a crucial role (as can be seen by comparing compounds **1-6**, Table 1) and this analysis confirms that the thiazolinethione (R_1 = S) is preferred.

- 5-aryl regioisomers are systematically less potent than the corresponding 4-aryl analogs. This suggests that the rather constrained binding site of human IDO1 is sensitive to the relative positions of the sulfur atom (potentially coordinating the iron of the heme cofactor) and the aromatic ring engaged in π-π and van der Waals interactions in the hydrophobic binding pocket.

- In contrast with what was anticipated, pyridine isosteres (**1o** and **1p**) did not show increased inhibition potency.

Overall, substitution on the phenyl ring does not significantly increase inhibition potency of **1**, with the notable exception of 4-Cl (**1m**) and 4-Br (**1n**), which are more potent inhibitors of IDO1. The activity of *ortho*-substituted analogs is conserved (*e.g.* **1c** *vs* **1f** and **1l**) suggesting that this position can be substituted, as is the case for 4-aryl-1,2,3-triazoles.[23] This further suggests that the bioactive conformation is non-coplanar. In the crystal structure of **1**, the dihedral angle between the phenyl and thiazolinethione rings is close to 40°.[15] This non-coplanar conformation, retained with *ortho* substituents, diminishes conjugation between the two aromatic rings, so that electronic effects, in particular resonance effects, due substituents on the phenyl ring, should not significantly affect charge distribution

(and acidity) of the thiazolinethione moiety. Therefore, steric and/or hydrophobic effects of the substituents probably play a more important role. Small substituents are tolerated (%Inh halogens (**1m**, **1n**) > Me (**1h**) > OMe (**1i**) > CN (**1j**)) consistent with a small sterically constrained pocket A in IDO1. This binding pocked being composed of apolar residues, hydrophobic substituents are favored, with 4-Me (**1h**) and 4-Br (**1n**) being the best in the series. *A posteriori* docking of 4-Br (**1n**) confirmed that the bromide substituent could be accommodate into the binding site of IDO1 and close proximity of the thiol group of Cys129 and the *para* position of the aromatic ring (Br···S : 3.3 Å; C···S : 3.0 Å) might indicate a sulfur-π interaction (see supporting information).

In summary, docking studies of 4-phenylthiazolinethione, **1**, on human IDO1 suggested that a complexation of the heme iron is possible, and is accompanied by hydrophobic interactions and π-π stacking. Molecular modelling further delineated the structural variations that the enzyme pocket should tolerate. In order to obtain a rudimentary SAR, we decided to prepare a number of derivatives bearing simple electron donating and withdrawing groups. Among the more intriguing possibilities, we identified the possibility of hydrogen bonding by an acceptor in the para position of the aryl group. Furthermore, the orientation of the aryl substituent could be modified by using the 5-aryl derivatives.

To reach our goal, synthetic routes had to be adapted and optimized to afford the desired substituted 4- and 5-arylthiazolinethiones. Biological evaluation of the small library of molecules shows that 5-aryl regioisomers are systematically less potent than the corresponding 4-aryl derivatives. Despite our previously stated rationale, substitution on the phenyl ring does not significantly increase inhibition potency compared to lead compound **1**, except for 4-Me (**1h**), 4-Cl (**1m**) and 4-Br (**1n**) derivatives, which are more potent inhibitors of IDO1.

Our study suggests that the 4-arylthiazolinethione remains an interesting scaffold for the design of IDO1 inhibitors but that the potential for substitution on the phenyl ring is limited. Further directions for optimization of this series include the possibility to reach the second binding site (pocket B) of the enzyme. This could potentially be achieved by substituting **1** at the nitrogen atom of the thiazolinethione. We are now investigating this challenging possibility. Co-crystallization of the purified IDO1 enzyme with **1** and/or **1m** is also an option to confirm our docking hypothesis and further guide the design of new analogues.

Acknowledgments

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Supplementary Material

Supplementary Material contains: experimental procedures and characterization of all synthesized compounds; docking result for **1n**; dose-response curves IC_{50} (determination) for compounds **1**, **1h**, **1m,** and **1n**.

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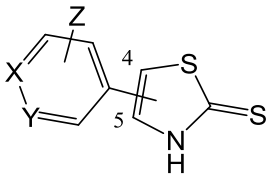

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