Marine Natural Products and Other Derivatives as Potent Indoleamine 2,3-Dioxygenase Inhibitors

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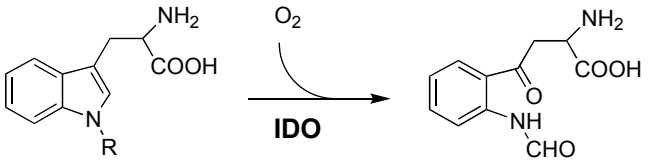
Abstract: Cancer cells are able to elaborate enzymatic mechanisms allowing tumors to resist or escape imune rejection. Among the enzymes involved, indoleamine 2,3-dioxygenase (IDO), an intracellular enzyme that initiates the first and rate-limiting step of tryptophan breakdown along the kynurenine pathway, has emerged as a promising molecular target for the development of new immunotherapeutic anticancer agents. This review summarizes the synthesis and IDO activities of the different classes of marine and other inhibitors reported so far.

Keywords: Indoleamine 2,3-dioxygenase, IDO inhibitors, marine natural compounds.

INTRODUCTION

Indoleamine 2,3-dioxygenase (IDO); EC 1.13.11.42) is an intracellular monomeric heme-containing enzyme that initiates the first and rate limiting step of mammalian tryptophan catabolism in the kynurenine pathway [1] Scheme (**1**).



L-tryptophan, R = H
1-MT, R = Me

N-formylkynurenine

Scheme (1).

This biosynthetic route leads to a series of biologically active metabolites including neurotransmitters serotononin and melatonin, excitoxin, quinolinic acid, N-methyl-D-aspartate (NMDA) receptor antagonist, kynurenic acid and ultimately the production of nicotinamide adenine dinucleotide (NAD) [2-4]. IDO is active when the heme iron is in the ferrous (Fe^{2+}) form and inactive when it is in the ferric (Fe^{3+}) form [5,6]. Recently, IDO was suggested to be involved in the capacity of many tumors to actively suppress a potentially effective immune response [7-10]. As a result, IDO arises as an important drug target to discover a new therapeutic approach to cancer treatment and there has been considerable interest in evaluating the potential of IDO inhibitors.

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Since the most frequently used, 1-methyltryptophan (1-MT), was identified as a weak competitive inhibitor of the enzyme (Ki = 34 μM) in the early 1990s, [11] few structural classes of compounds were described to be IDO inhibitors [12]. A previous account by Di Pucchio *et al.,* was devoted to patented lead compounds, [13] the present review will update and enlarge the topic, focusing on synthesis and biological evaluation of IDO inhibitors.

IDO INHIBITORS

Marine Natural Compounds and Analogues

Until a few years ago, the best known IDO inhibitor including β-carbolines [14] and 3-amino-2-naphthoic acid [15] displayed affinities in the micromolar range. In 2006, as a result of a screening of a library of marine invertebrate extracts for their ability to inhibit purified recombinant human IDO *in vitro*, Andersen's group identified the new polyketides annulin C **1** and the known compounds annulin A **2** and annulin B **3** as sub-micromolar IDO inhibitors in the Northeastern Pacific hydroid *garveia annulata* crude extract Fig. (**1**) [16].

A second group of compounds including the new metabolites 2-hydroxygarveatin E **4** and garveatin E **5**, as well as the known compounds garveatin A **6**, garveatin C **7** and 2-hydroxygarvin **8** issued from the same organism was also found, but to a lesser extent, to inhibit IDO. The Ki values for *in vitro* inhibition of IDO by the *G annulata* polyketides listed in Table **1** were determined by continuous spectrophotometric analysis.

Annulin A, B and C revealed to be inactive in a yeast-based cellular assay developed by the same group, [17] suggesting poor cell penetration. Two other compounds issued from marine source namely, imidocarbonimidic diamine, N-methyl-N'-9-phenanthrenyl-monohydrochloride **9** and caulerpin **10** were also identified as 1 μmolar order IDO inhibitors by using this yeast-based IDO inhibition assay Fig. (**2**).

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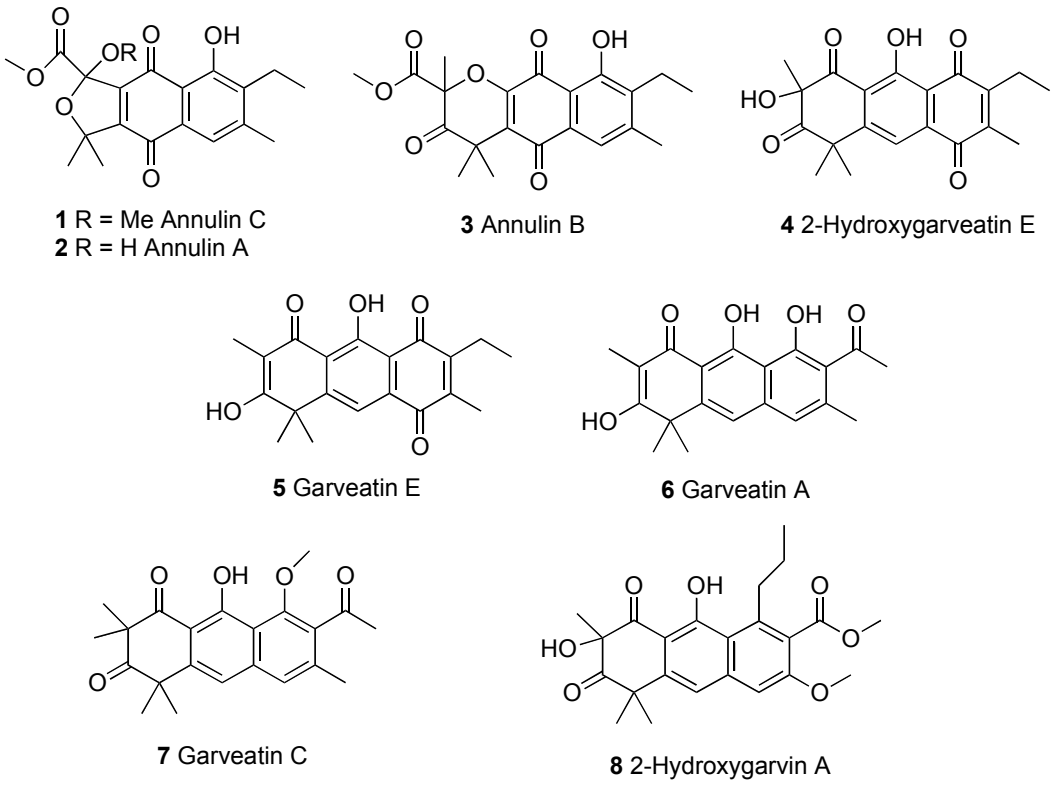


Fig. (1). IDO polyketides inhibitors issued from the marine hydroid *Garveia annulata*.

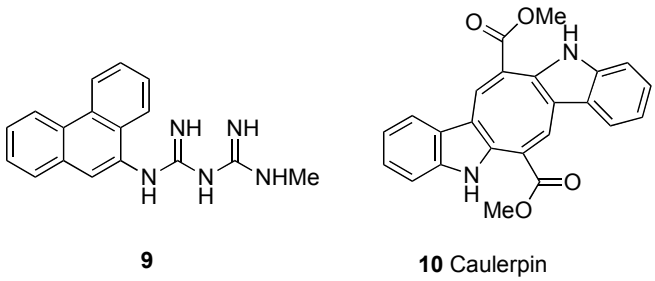
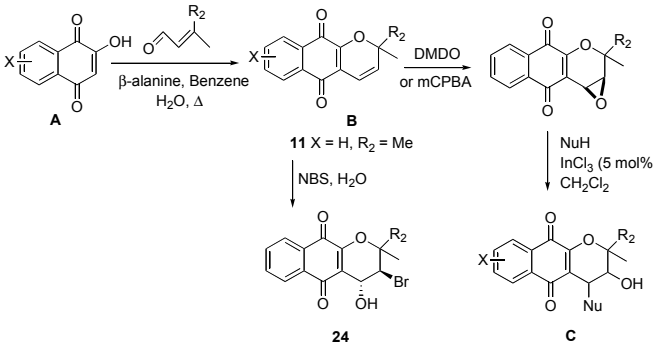


Fig. (2). IDO inhibitors identified with a target-based screen in yeast.

Table 1. ***In Vitro*** **Inhibition of IDO by** ***G. annulata*** **Metabolites**

	Kį (μM)
Annulin C (**1**)	0.14
Annulin A (**2**)	0.69
Annulin B (**1**)	0.12
2-hydroxygarveatin E (**4**)	1.4
garveatin E (**5**)	3.1
garveatin A (**6**)	3.2
garveatin C (**7**)	1.2
2-hydroxygarvin A (**8**)	2.3
1-MT	6.6

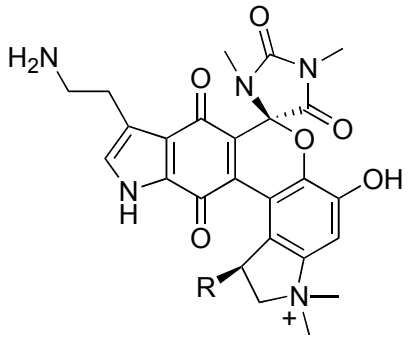
A medicinal chemistry program that generated a series of synthetic IDO inhibitory pyranonaphtoquinones was inspired by annulin B [18]. In this study devoted to compounds containing naphtoquinone as the key pharmacophore, the authors described the synthesis and characterization of a new class of pyranonaphtoquinones. In a first step, naphtoquinones **A** were converted to pyranonaphtoquinones **B** via a one-pot 6Π electrocyclization reaction. After epoxidation, derivatization of the pyran ring was accomplished by nucleophilic substitution of the epoxide leading to cis and trans diastereoisomers **C** which were separated by column chromatography (Scheme **2**). The IDO inhibitory potency of the more interesting compounds, reported in Table **2**, revealed compounds with IC_{50} of low nanomolar values.



Scheme (2).

Nevertheless, analysis of the most potent compounds **15**, **16** and **21** in the same cell-based assay to analyze menadione (2-methyl-1,4-naphtoquinone known also as vitamin K3) for which IDO has been proven to be an essential target for the antitumor activity, [19] showed an attenuation of their activity versus the isolated enzyme assay.

The same screening by Andersen's group led also to isolate the novel alkaloid exiguanine A **25** from a MeOH extract of the sponge *Neopetrosia exigua* collected in Papua New Guinea [20] Fig. (**3**).



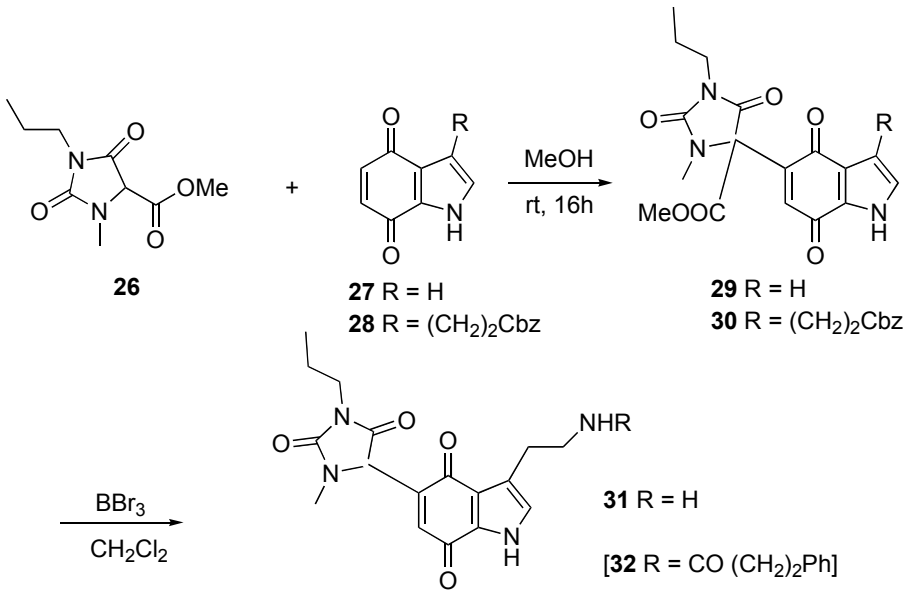
25 R = H Exiguamine A
32 R = OH Exiguamine B

Fig. (3). IDO inhibitors issued from the marine sponge *Neopetrosia exigua*.

The Ki of this new metabolite (41 nM) making it one of the more potent IDO inhibitor, prompted them to study different analogues [21]. The compounds **29-31** were prepared on the basis of a coupling reaction between hydantoin **26** and indolequinones **27** or **28** according to the synthesis reported in Scheme (**3**), (Table **3**).

Table 2. IC_{50} Values of Pyranonaphtoquinone Derivatives IDO Inhibitors

Structures (Scheme 2)		X	R_2	Nu	IC_{50} (μM)
B	**11**	H	Me		0.214
	12	H	CO_2Me		0.247
	13	6-OH	Me		0.190
	14	8-OMe	Me		0.933
	15	9-OH			0.121
C	**16**	H		$PhCH_2NH$ cis	0.055
	17	H		$PhCH_2NH$ trans	0.252
	18	H		$CH_2=CHCH_2NH$ cis	0.186
	19	H		$CH_2=CHCH_2NH$ trans	0.183
	20	H		$Me(CH_2)_3NH$ cis	0.130
	21	H		$Me(CH_2)_3NH$ trans	0.087
	22	H		Morpholinyl trans	0.361
	23	H		OMe cis	0.976
	24	H		3-Br	0.519



Scheme (3).

This procedure was adapted to prepare compound **32**. The ability of the synthetic analogues to inhibit recombinant human IDO are provided in Table **2**. All the analogues are slightly less active than the natural compound exiguamine with Ki values ranging from 190 to 420 nM.

Table 3. ***In Vitro*** **Inhibition of IDO by Exiguamine and Analogues**

	Ki nM)
Exiguamine A (**25**)	41
27	190
29	420
30	260
31	200
32	260

Two years later, in order to confirm their hypothesis to assemble Exiguamine A along biosynthetic lines, Andersen and co-workers proposed a mimetic synthesis of the natural product which also led them to consider the formation of an hydroxylated derivative **33** (Fig. (**3**)) [22]. They were able to independently isolate this new compound which they called exiguamine B from *N-exigua* and to show it to inhibit IDO with a Ki value of about 80 nM.

From a third extract issued from laboratory cultures of the fungus Plectophaerella cucumerina from marine sediments collected at -100 m depth in Barkley sound, British Columbia, Andersen's group identified the novel alkaloids plectosphaeroic acids A **34**, B **35** and C **36** as *in vitro* inhibitors of IDO (Fig. (**4**)) [23]. These three compounds all inhibited purified recombinant human IDO and exhibited identical IC_{50} of 2 μM order.

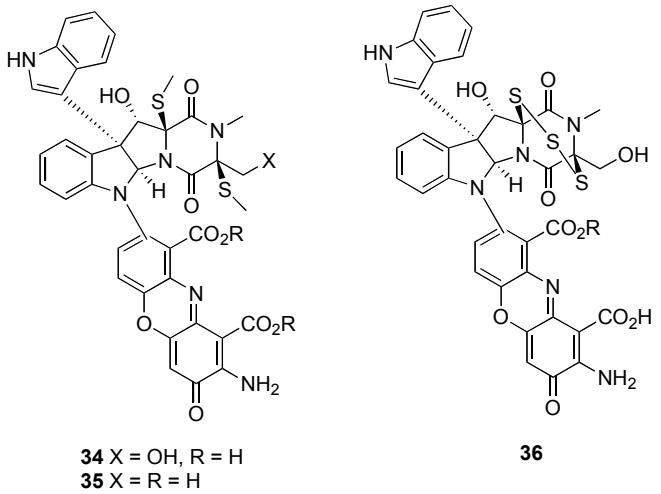


Fig. (4). IDO inhibitors issued from laboratory cultures of the fungus *Plectosphaerella cucumerina* obtained from marine sediments.

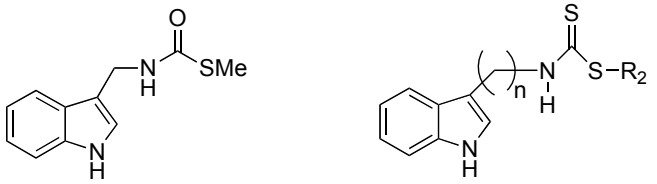
Other Compounds

In 2006, a screening of commercially available indole-based molecules by Malachowski, Muller, Pendergast and co-workers, revealed the natural phytoalexin brassinin **37** to be a moderately active IDO competitive inhibitor (Ki = 97.7 μM) [24] Fig. (**5**).

In order to undertake a structure-activity relationship study, different brassinin dithiocarbamate analogues were synthesized by adding an amine to carbon disulfide at 0 °C, stirring for 1 h, and then adding an alkyl halide. Among these compounds, analogues **38-41** with Ki ranging from 12 to 20 μM were more potent inhibitors than brassinin.

Later on, after the first crystal structure of IDO was reported [25] and taking account that 4-phenylimidazole (4-PI) was identified as a weak non-competitive inhibitor of the

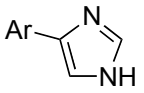
enzyme, [26] the same group used computational docking experiments guided design and investigated 4-phenylimidazole analogues [27] Fig. (**6**).



37 brassinin

38 n = 1, R_2 = CH_2Ph
39 n = 2, R_2 = CH_2Ph
40 n = 2, R_2 = CH_2Napht
41 n = 2, R_2 = CH_2-4-Pyr

Fig. (5). IDO inhibitors Brassinin and analogues.



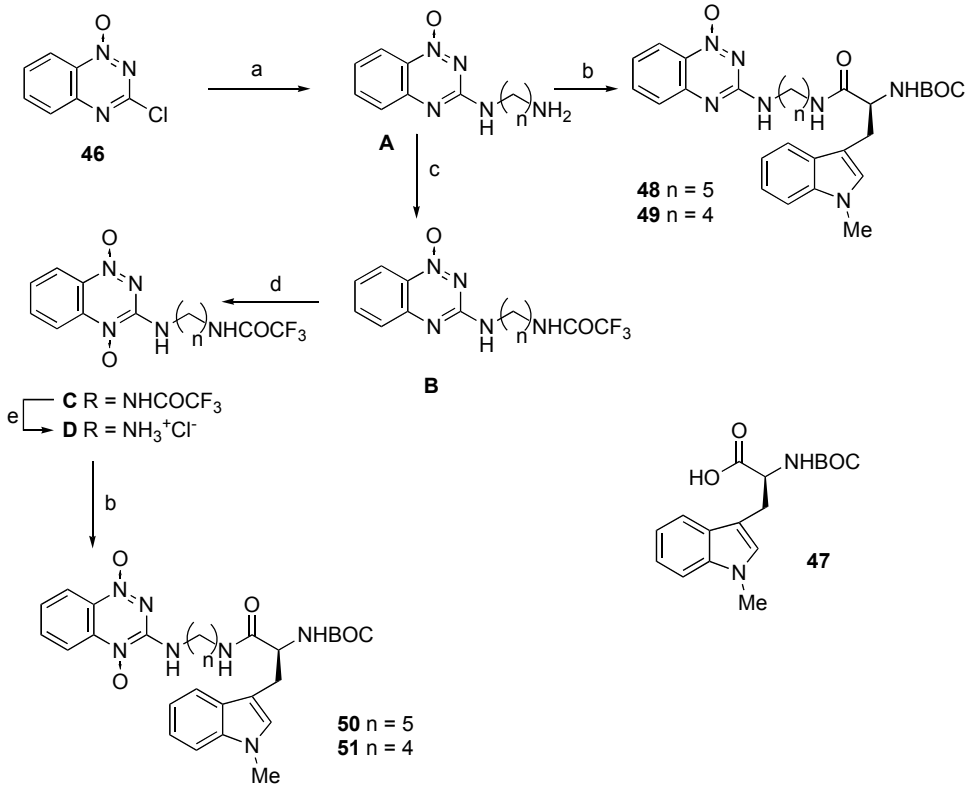
42 Ar = 2-OH-Ph
43 Ar = 2,6-(OH-)$_2$Ph
44 Ar = 3-SH-Ph
45 Ar = 4-SH-Ph

Fig. (6). Phenylimidazole-derived IDO inhibitors.

The most interesting compounds **42-45** (IC_{50} of 4.8, 5.3, 7.6 and 7.7 μM respectively) were obtained either by de novo imidazole ring synthesis involving α-bromoketones with formamide for the two first ones or through Suzuki coupling reaction between 4-bromoimidazole and thioether substituted phenyl derivatives for the two others.

In 2008, a Japanese group, starting from tirapazamine (TPZ, 3-amino-1,2,4-benzotriazine-1,4-dioxide) a hypoxic cytotoxin under II/III clinical trials in combination with radiotherapy and cisplatin-based chemotherapy, and **1-MT**, designed and synthesized the **1- MT**-TPZ hydrids **48-51** defined as hydridization of **1-MT** with TPZ or TPZ-monoxide (Scheme **4**) [28].

The compounds were prepared from the chloride **46** which was first reacted with alkyldiamines to give the corresponding 3-(aminoalkylamino) monoxides **A**. These intermediates were either coupled with BOC-N-1-MT **47** to yield TPZ-monoxide hydrids 48 and 49, or protected with the trifluoroacetyl group to give trifluoroacetamide derivatives **B**. After previous conversion of **B** into the dioxide compounds **C** and deprotection under basic conditions, the resulting **D** compounds were coupled with **47** to give the TPZ-dioxide hydrids **50** and **51**. Compounds **48-51** have been shown to be potent uncompetitive IDO inhibitors with the TPZ-monoxide hydrids **48** and **49** exhibiting the stronger inhibition (Ki values of about 80 μM). In addition, the TPZ-dioxide hydrids **50** and **51** have presented higher hypoxic-selective cytotoxicity than TPZ suggesting that they may act through their dual biological functions: first they function as hypoxic cytotoxins in hypoxic cells, and then are metabolized to their TPZ-monoxide hydrids which function as IDO inhibitors.



Scheme 4. (**a**) alkyldiamine, Et_3N, CH_2Cl_2, rt. (**b**) **47**, DIPCDI, HOBt, CH_2Cl_2, rt. (**c**) $(CF_3CO)_2O$, CH_2Cl_2, rt. (**d**) 35 % H_2O_2, $(CF_3CO)_2O$, CH_2Cl_2, rt. (**e**) 28 % NH_3 aq, MeOH, rt.

Another series of potent and competitive IDO inhibitors that strongly support the role of IDO in tumor progression has been discovered by Combs and co-workers [29]. They investigated 4-amino-1,2,5-oxadiazole-3-carboximidamides **A** possessing a hydroxyamidine chemotype as a key pharmacophore. These compounds were readily prepared in three steps starting from malononitrile **52** and via chloride **54** which was coupled to a variety of benzylamines and anilines (Scheme **5**).

All the compounds were tested *in vitro* for their ability to inhibit human IDO in the standard enzymatic assay and a HeLa cellular assay measuring kynurenine formation (Table **4**). Testing in mice **57**, which was chosen due to its improved physical properties compared to **58**, demonstrated decreased kynurenine levels by > 50 % in plasma and dose-dependent efficacy in mice bearing GM-CSF-secreting B16 melanoma tumors.

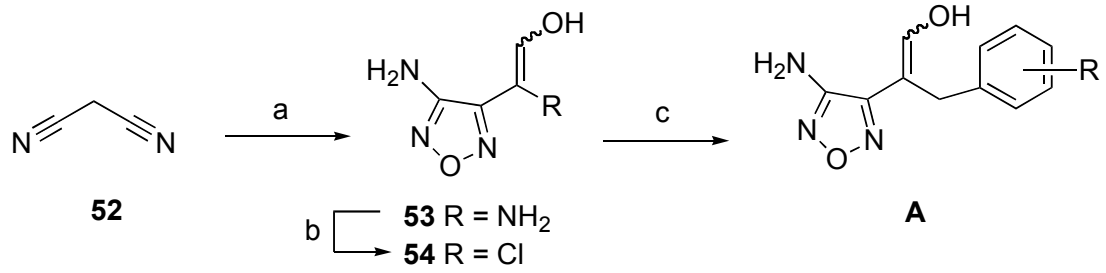
A structure-activity relationship for IDO inhibition including cellular activity was undertaken on a series of substituted benzylisothioureas and other functional substituted benzyl derivatives by Matsuno *et al*., [30]. The isothiourea compounds were obtained by alkylation of thiourea with the corresponding phenylalkyl halide. Evaluation of all compounds led to the identification of sub-μM inhibitors, both of which suppressed kynurenine production in A431 cells, with the more potent compounds **59-65** being listed in Table **5**.

In order to ratiotionalize design of IDO inhibitors, Zoete, Michielin and co-workers have used the evolutionary docking algorithm EADock [31]. By pharmacophore-based lead design, they discovered three families of IDO inhibitors including benzothiazoles, phenylthiazoles and triazoles with one compound of each family (**66-68** respectively) exhibiting good result in the cellular assay Fig. (**7**).

They also used a fragment-based approach which led them to identify new low-molecular weight inhibitor scaffolds such as **69** and **70** and the three oxidized compounds **71-73** which showed IC_{50} of 200 to 500 nM in the enzymatic assay. In cellular assays, however, these compounds showed slightly worse results than reduced compounds **69** and **70** (Table **6**).

Recently, with the aim of defining novel IDO inhibitors, Dolusic *et al.,* undertook a virtual screening of very large libraries of commercially available chemicals including high-throughput docking which led them to discover compound **75** (with R = H) endowed with an inhibitory potency of the micromolar range (Scheme **6**) [32].

A complete SAR of a series of analogues of this compound based on the indol-2-yl ethanone scaffold **74-76** was realized by the same group [33]. Most of the compounds were prepared from esters (**77** R = OMe, OEt), or acyl chloride (**77** R = Cl) by using classical procedures. *In vitro* and *in vivo* biological activities have been evaluated, leading to compounds of moderate potential with IC_{50} values in the micromolar range in both tests.



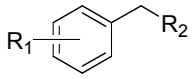
Scheme 5. (**a**) $NaNO_2$, 2N HCl, $NH_2OH.HCl$, then 10 N NaOH, D, 2 h. (**b**) $NaNO_2$, HCl, H_2O, 0 °C, 1.5 h. (**c**) aniline, Et_3N, EtOH, 0-25 °C.

Table 4. IDO Inhibition of 4-Amino-1,2,5-oxadiazole-3-carboximidamides A **in the Standard Enzymatic Assay and a HeLa Cellular Assay**

Compounds of Structure A (Scheme 5)	R	IDO (IC_{50} nM)	HeLa (IC_{50} nM)
55	3-Cl	86	19
56	3-Br	73	17
57	3-Cl, 4-F	67	19 (46)*
58	3-Br, 4-F	59	12

*Murine cellular B16 assay IC_{50} (nM).

Table 5. IDO Inhibition of Benzylthiourea and Benzylthiol Derivatives

	R_1	R_2	HX	IDO (IC_{50} µM)	A431 (IC_{50} µM)*
59	3-Cl	$SC(=NH)NH_2$	HCl	4.6	1.4
60	4-Cl	$SC(=NH)NH_2$	HCl	2.2	0.6
61	4-CF_3	$SC(=NH)NH_2$	HCl	2.6	1.2
62	2,4-Cl_2	$SC(=NH)NH_2$	HBr	0.4	1.1
63	4-Cl	SH	HCl	3.2	1.1
64	4-F	SH	HCl	1.8	1.9
65	3,4-Cl_2	SH	HCl	0.1	1.1

* Kynurenine production in A431 cells.

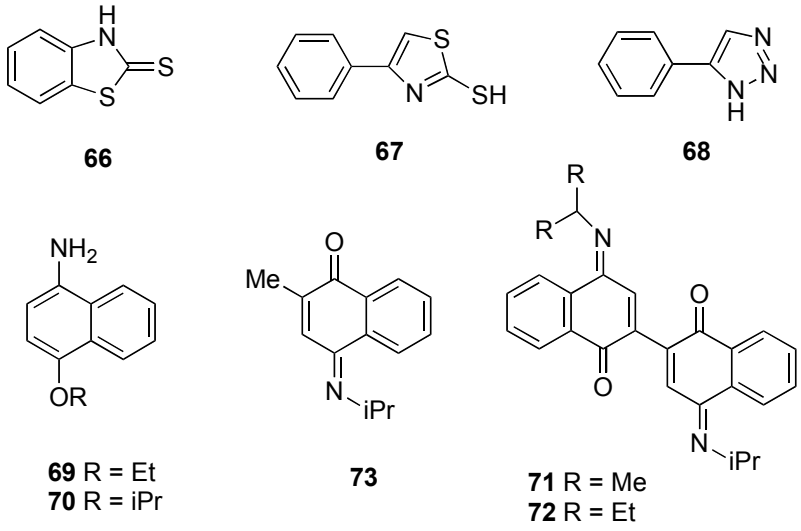
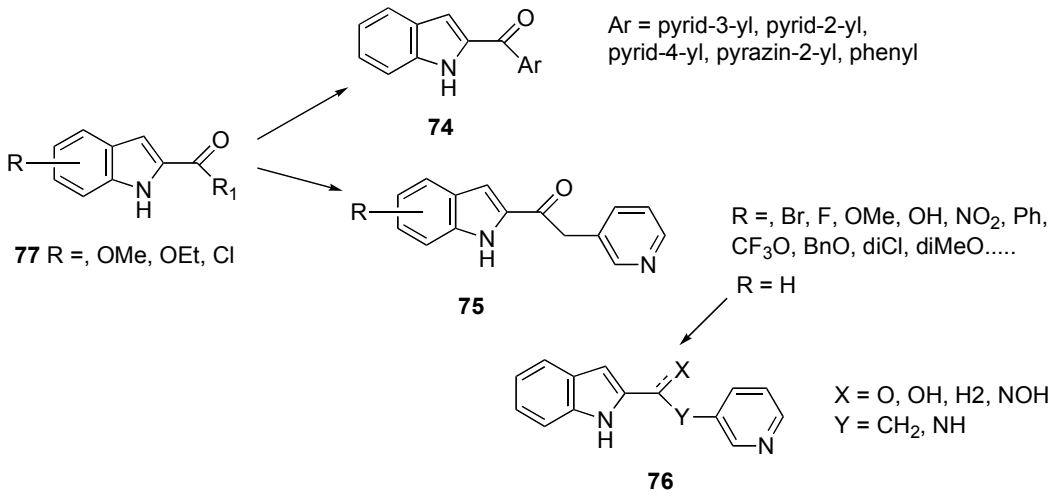


Fig. (7). IDO inhibitors designed by using the evolutionary docking algorithm EADock.

Table 6. Cellular and Enzymatic Assays for Human IDO Inhibition.

	Enzymatic hIDO (IC_{50} µM)	Cellular hIDO (IC_{50} µM)
66	50	7
67	50	4
68	60	70
69	16	50
70	2.5	12.5
71	0.45	25
72	0.52	50
73	0.20	> 50



Scheme (6).

CONCLUSION

Since the discovery of IDO as a relevant target in cancer treatment, a large number of compounds, including marine metabolites, have been reported to exhibit inhibitory properties. Although a unique compound, **1-MT** has reached clinical trials, it can be hope that continuing effort in screening or design may provide valuable novel medicinal therapeutic agents.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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Declared none.

PATIENT CONSENT

Declared none.

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