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Indole-pyridinyl-ethanones as Novel Inhibitors of Indoleamine 2,3-Dioxygenase (IDO), a Promising Target for Anti-Cancer Immunotherapy

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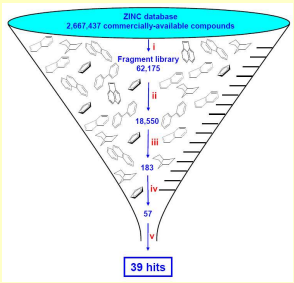
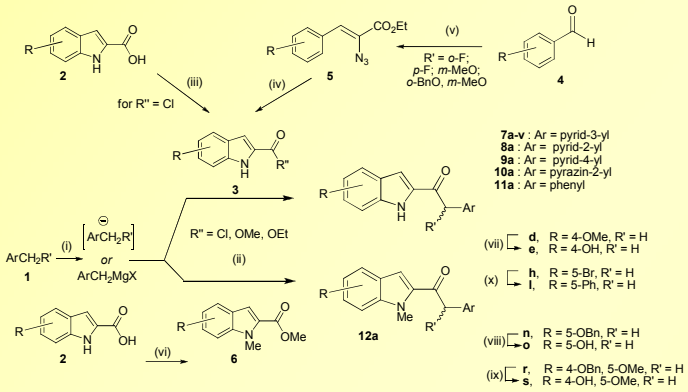
Introduction. Immunotherapy is a promising novel strategy for cancer therapy. However, this approach showed a limited efficacy *in vivo* because cancer cells can develop mechanisms allowing tumors to resist or escape immune rejection.
IDO (EC 1.13.11.52), a heme dioxygenase, is expressed constitutively in many human tumors and its role in a tumoral immune resistance mechanism has been proved,[1] justifying the interest in IDO inhibitors.[2]
Aim of the Work. We sought to develop a novel series of IDO inhibitors starting with a virtual screening of a database of commercially-available compounds.



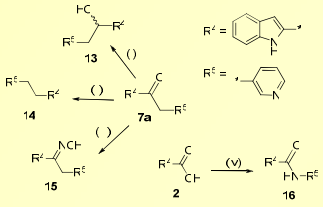
Virtual screening. Based on recent results such as structural findings[3] and rational design of IDO inhibitors,[4] we applied virtual screening of the ZINC database (*http://zinc.docking.org*) for the discovery of new inhibitors (Figure 1). The most promising candidates were purchased and tested *in vitro*. 1-(1*H*-Indol-2-yl)-2-pyridin-3-yl-ethanone (**7a**; IC_{50} = 65 µM) was selected for pharmaco-modulation (Schemes 1 and 2 and Table 1).

Figure 1.
IDO Virtual screening flowchart. (i) fragment library, (ii) goldscore > 50, (iii) Cscore ≥ 4, (iv) visual analysis and selection, (v) *really* commercially-available.

Synthesis



Scheme 1. General synthetic scheme for indol-2-yl ethanones.[5] Reagents and conditions: (i), LDA, THF / hexanes, -78 to 0°C, 1 h; (ii) THF / hexanes, 0°C to rt, 16-24 h; (iii) $SOCl_2$, Δ, 15 min; (iv), hexane, 200°C (µW), 5 min; (v), $N_3CH_2CO_2Et$, NaOEt, EtOH, -10 to 4°C, 1.5 - 20 h; (vi), MeI, K_2CO_3, DMF, 80°C, 5 days; (vii), $AlCl_3$, CH_2Cl_2, 0°C to r. t, 24 h; (viii), HCO_2NH_4, Pd black, MeOH, r. t, 1 h; (ix) H_2 (1 atm), 3% Pd/C, EtOH, r. t, 45 min; (x) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, EtOH/toluene 1/1, Δ, 20 h.



Scheme 2. Synthesis of compounds bearing different linker groups. Reagents and conditions: (i) HCO_2NH_4, Pd black, MeOH, r. t, 3 days; (ii), H_2NNH_2, KOH, $(CH_2OH)_2$, µW, 1h then aq. NH_4Cl; (iii), $NH_2OH{\cdot}HCl$, pyridine, EtOH, µW (120°C), 30 min; (iv), $SOCl_2$, Δ, 15 min then 3-aminopyridine, DIPEA, THF, 0°C -> r. t, 30 min.

Table 1. Biological evaluation

compound	R	R'	enzymatic assay IC_{50} (µM)	cell assay inh. % @ 20 µM
7a	H	H	65	13
7b	3-Br	H	>100	NI
7c	4-F	H	153	12
7d	4-OCH_3	H	58	NI
7e	4-*OH*	H	83	NI
7f	5-F	H	36	24
7g	5-*Cl*	H	25	24
7h	5-Br	H	18	NI
7i	5-CH_3	H	87	21
7j	5-OCH_3	H	49	-
7k	5-NO_2	H	>100	NI
7l	5-Ph	H	96	NI
7m	5-OCF_3	H	13	12
7o	5-*OH*	H	37	NI
7p	6-F	H	43	NI
7q	7-OCH_3	H	82	-
7r	4-OBn, 5-OCH_3	H	>100	NI
7s	4-OH, 5-OCH_3	H	63	11
7t	4,6-di*Cl*	H	139	NI
7u	4,6-di(OCH_3)	H	45	toxic
7v	H	CH_3	141	NI
8a	H	H	37	10
9a	H	H	>100	NI
10a	H	H	26	NI
11a	H	H	29	NI
12a	H	CH_3	34	NI
13	H	H	>100	NI
14	H	H	>100	NI
15	H	H	>100	25
16	H	-	94	NI

NI = no inhibition

Conclusion. The synthesis and SAR of a novel series of IDO inhibitors are described. Starting from the lead compound **7a** (IC_{50} = 65 µM) identified through a virtual screening procedure, up to a 5-fold improvement in *in vitro* potency could be achieved by introducing small substituents in the 5- and 6-positions of the indole nucleus. Most modifications of the aromatic moieties are tolerated. On the contrary, the presence of an iron chelating group on the linker seems to be mandatory, as corroborated by the docking experiments (Fig. 2). A number of compounds are also moderately active in the *in vivo* assay, thus opening possibilities for further pharmacological evaluation.

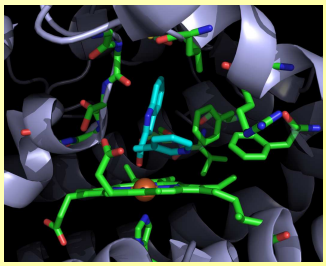


Figure 2. Docking of compound**7a** inside the IDO active site showing interaction of the carbonyl oxygen with the heme iron

References. [1] Uyttenhove, *C. et al, J. Nat Med* **2003**, *9*, 1269-1274; [2] Macchiarulo *et al, Inflamm. Res.* **2009**, *37*, 219-229; [3] Sugimoto H. *et al, PNAS* **2006**, *103*, 2611-2616. [4] Röhrig, U. *et al, J. Med. Chem.* **2010**, *53*, 1172-1189; [5] Sundberg, R. *et al, J. Org. Chem.* **1978**, *43*, 4859-4865.

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