Electronic Supplementary Material (ESI) for MedChemComm.

In silico-driven multicomponent synthesis of 4,5- and 1,5-disustituted imidazoles as indoleamine 2,3-dioxygenase inhibitors

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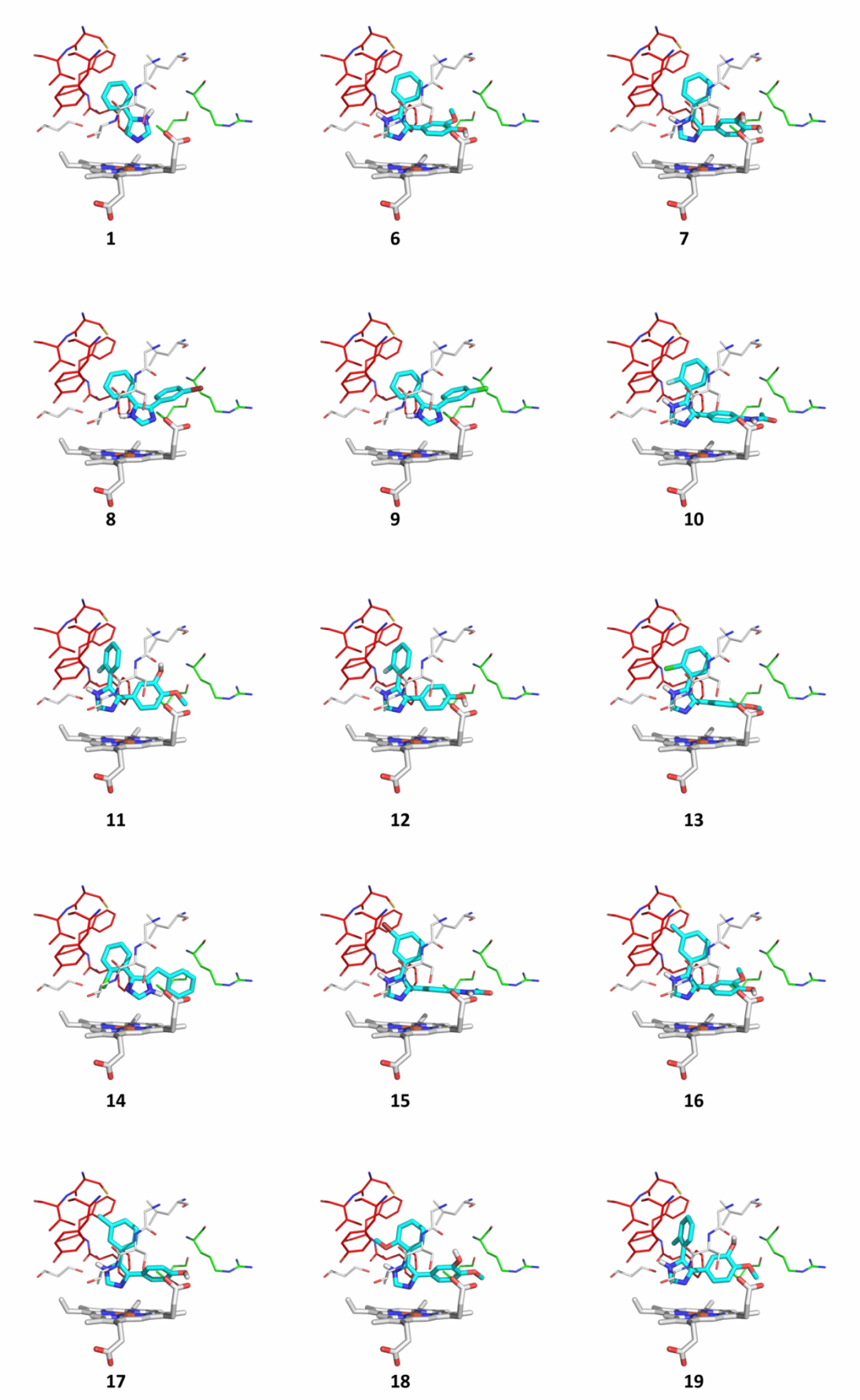
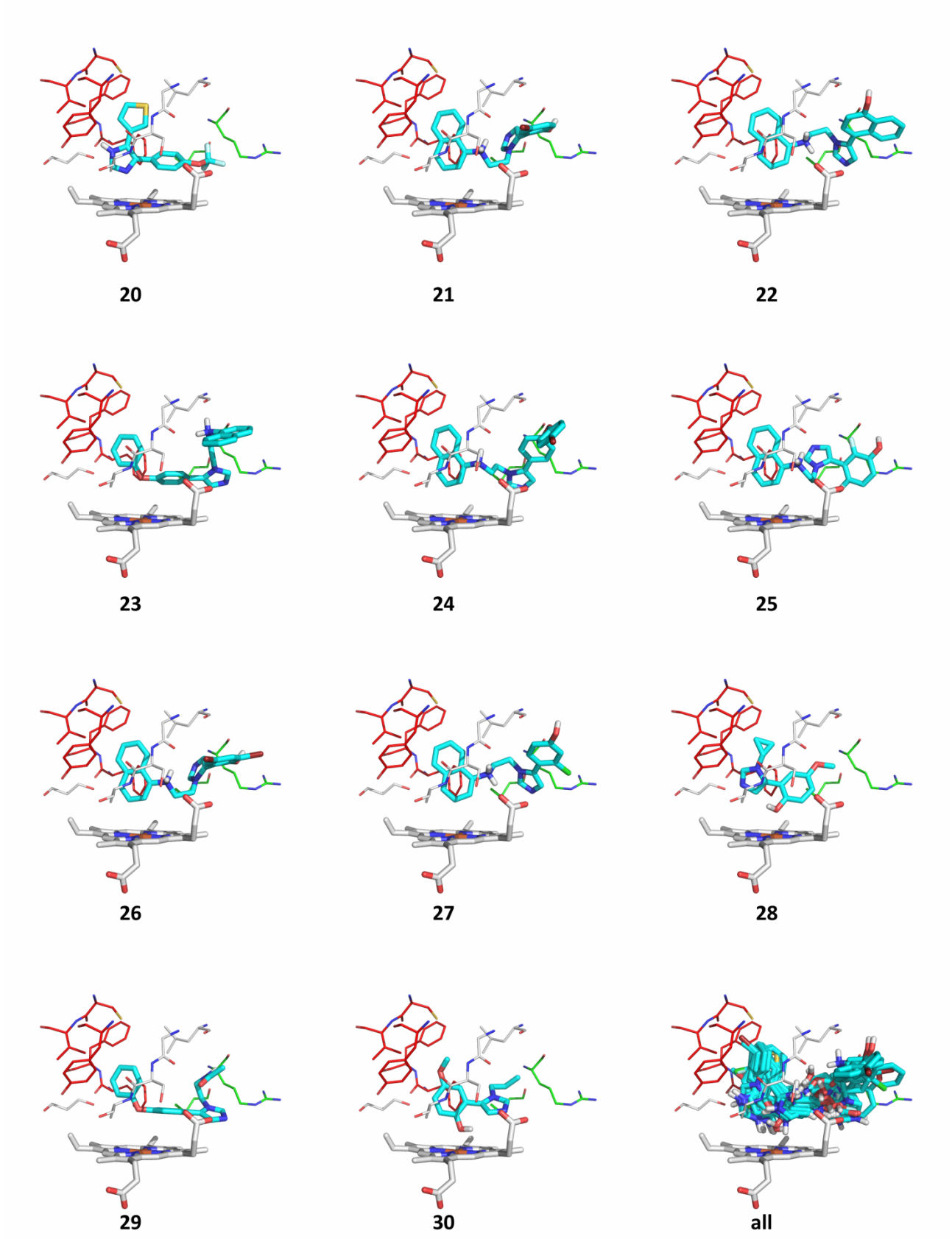


Fig. 1S. Docked pose of the synthesized compounds (**1**, **6**-**30**). Docked compounds are depicted as cyan sticks, pocket A as red lines and pocket B as green lines.

Fig. 1S. Docked pose of the synthesized compounds (continue).



General procedure for the synthesis of formamides a-g.

To a solution of aldehyde (1 equiv) in acetonitrile and toluene, formamide (2.5 equiv) and TMSiCl (1.1 equiv) are added. After stirring at 50 °C for four hours under a nitrogen atmosphere, *p*-toluensulphinic acid (1.5 equiv) is added. The reaction mixture is stirred at 50 °C for 24 hours. The reaction is cooled to room temperature, diluted with Et_2O and washed with water (1x). The organic phase is dried over sodium sulphate and evaporated to give a solid. TBME is added and the suspension is cooled to 0 °C in order to promote the precipitation. The precipitated white solid is collected by filtration using a Buchner funnel and rinsed with TBME (x 3). The obtained material is used in the next step without further purification.

***N*-(Phenyl(tosyl)methyl)formamide (a).** White solid. Yield 60%. mp 156-157 °C. ν_{max}/cm^{-1} 3337, 2977, 1593, 1514, 1454, 1319, 1148, 816, 696. ^1H NMR δ_H (300 MHz; DMSO-d6) 7.97 (s, 1 H), 7.71 (d, *J* = 7.9 Hz, 2 H), 7.56-7.53 (m, 2 H), 7.46-7.38 (m, 5 H), 6.39 (d, *J* = 10.7 Hz, 1 H), 2.40 (s, 3H). ^{13}C NMR δ_C (75 MHz; DMSO-d6) 160.8, 145.4, 133.9, 130.8, 130.2, 130.0, 129.7, 128.8, 128.7, 70.7, 21.7.

***N*-((2-Fluorophenyl)(tosyl)methyl)formamide (b)**. White solid. Yield 70%. mp 149-150 °C. ν_{max}/cm^{-1} 3359, 3274, 2961, 2880, 1591, 1513, 1318, 1147, 817, 764. ^1H NMR δ_H (300 MHz; $CDCl_3$) 8.63 (d, *J* = 10.4 Hz, 1 H), 8.07 (s, 1 H), 7.65 (d, *J* = 8.2 Hz, 2 H), 7.53 (m, 1 H), 7.35 (m, 1 H), 7.25 (d, *J* = 8.2 Hz, 2 H), 7.15 (t, *J* = 7.8 Hz, 1 H), 6.99 (t, *J* = 7.8 Hz, 1 H), 6.60 (d, *J* = 10.4 Hz, 1 H), 2.37 (s, 3 H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 160.9 (d, *J* =248.5 Hz), 160.4, 145.6, 133.2, 131.7 (d, *J* = 8.5 Hz), 130.2, 129.8, 129.4, 124.5 (d, *J* = 3.4 Hz), 118.0 (d, *J* = 13.1 Hz), 115.7 (d, *J* = 21.7 Hz), 65.2, 21.8.

***N*-((2-Chlorophenyl)(tosyl)methyl)formamide (c).** White solid. Yield 61%. mp 128-129 °C. ν_{max}/cm^{-1} 3367, 2968, 2919, 1595, 1506, 1480, 1306, 1143, 1085, 814. ^1H NMR δ_H (300 MHz; $CDCl_3$) 8.10 (s, 1 H), 7.71-7.57 (m, 3 H), 7.31-7.25 (m, 5 H), 6.98 (d, *J* = 9.9 Hz, 1 H), 2.38 (s, 3 H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 160.6, 145.9, 135.4, 133.2, 131.1, 130.0, 129.9, 129.6, 129.4, 128.7, 127.5, 67.0, 21.8.

***N*-((3-Bromophenyl)(tosyl)methyl)formamide (d).** White solid. Yield 31%. mp 138-145 °C. ν_{max}/cm^{-1} 3331, 2970, 2412, 1928, 1666, 1511, 1318, 1147, 580. ^1H NMR δ_H (300 MHz; CD_3OD) 8.08 (s, 1 H), 7.71 (d, *J* = 7.5 Hz, 2 H), 7.65 (s, 1 H), 7.59 (d, *J* = 7.2 Hz, 1 H), 7.46 (d, *J* = 7.2 Hz, 2 H), 7.32 (t, *J* = 7.2 Hz, 1 H), 6.33 (s, 1 H), 2.44 (s, 3 H). ^{13}C NMR δ_C (75 MHz; DMSO-d_6) 160.8, 145.6, 133.7, 133.5, 132.9, 132.6, 130.9, 130.2, 129.2, 129.0, 122.1, 70.0, 21.7.

***N*-(*m*-Tolyl(tosyl)methyl)formamide (e)**. Yellow solid. Yield 42%. mp 88-89 °C. ν_{max}/cm^{-1} 3530, 2868, 1685, 1318, 1145, 815, 580 cm-1.^1H NMR δ_H (300 MHz; DMSO-d6) 9.73 (d, *J* = 10.5 Hz, 1 H), 7.94 (s, 1 H), 7.71 (d, *J* =7.8 Hz, 2 H), 7.42 (d, *J* = 7.8 Hz, 2 H), 7.35-7.26 (m, 5 H), 6.31 (d, *J* = 10.5, 1 H), 2.41 (s, 3 H), 2.31 (s, 3 H). ^{13}C NMR δ_C (75 MHz; CDCl3) 160.8, 145.6, 144.4, 138.6, 133.1, 130.7, 129.9, 129.8, 129.6, 128.7, 126.1, 71.1, 21.6, 21.3.

***N*-((2-Methoxyphenyl)(tosyl)methyl)formamide (f).** White solid. Yield 78%. mp 155-156 °C. ν_{max}/cm^{-1} 3316, 2981, 2842, 1595, 1495, 1468, 1316, 1258, 1235, 1143, 815, 759. ^1H NMR δ_H (300 MHz; $CDCl_3$) 8.17 (s, 1 H), 7.99 (d, *J* = 6.8 Hz, 1 H), 7.72-7.59 (m, 2 H), 7.40-7.30 (m, 2 H), 7.22-7.20 (m, 2 H), 6.95 (m, 1 H), 6.79-6.70 (m, 2 H), 3.59 (s, 3 H), 2.37 (s, 3 H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 160.5, 157.8, 145.1, 133.9, 131.3, 130.3, 129.5, 129.4, 121.0, 118.4, 111.3, 67.7, 55.7, 21.7.

***N*-(*o*-Tolyl(tosyl)methyl)formamide (g).** White solid. Yield 63%. mp 134-135 °C. ν_{max}/cm^{-1} 3343, 2967, 2877, 1595, 1511, 1490, 1301, 1142, 811, 756. ^1H NMR δ_H (300 MHz; $CDCl_3$) 8.05 (s, 1 H), 7.71 (d, *J* = 8.2 Hz, 2 H), 7.55 (d, *J* = 7.1 Hz, 1 H), 7.31-7.21 (m, 4 H), 7.17 (d, *J* = 7.1 Hz, 1 H), 6.64 (d, *J* = 10.4 Hz, 1 H), 2.40 (s, 3 H), 2.33 (s, 3 H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 160.2, 145.6, 138.3, 133.5, 130.9, 129.9, 129.3, 129.0, 127.8, 127.3, 126.6, 66.6, 21.8, 19.7.

General procedure for the synthesis of substituted TosMIC derivatives h-n.

To a solution of formamide **a-g** (1 equiv) in THF, $POCl_3$ (2 equiv) is added under a nitrogen atmosphere at room temperature. The reaction is cooled to -10 °C and TEA (6 equiv) is added dropwise. The reaction is stirred at -10 °C for one hour and then quenched with $NaHCO_3$ at 0 °C. After reaching room temperature, the reaction is diluted with EtOAc, washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated. The crude material is purified by gravimetric column.

1-((Isocyano(phenyl)methyl)sulfonyl)-4-methylbenzene (h). Yellow solid. Yield 65%. mp 128-129 °C. ν_{max}/cm^{-1} 2951, 2130, 1592, 1489, 1454, 1331, 1151, 816, 722, 698. ^1H NMR δ_H (300 MHz; $CDCl_3$) 7.59 (d, *J* = 8.2 Hz, 2H), 7.48-7.29 (m, 7H), 5.62 (s, 1H), 2.45 (s, 3H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 166.2, 146.7, 130.8, 130.6, 130.1, 129.8, 128.8, 128.5, 126.7, 76.5, 21.9.

1-Fluoro-2-(isocyano(tosyl)methyl)benzene (i). Yellow solid. mp 216-217 °C. Yield 49%. ν_{max}/cm^{-1} 3064, 2939, 2140, 1594, 1492, 1340, 1159, 817, 709. ^1H NMR δ_H (300 MHz; $CDCl_3$) 7.73 (d, *J* = 8.5 Hz, 2H), 7.50-7.41 (m, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.12 (t, *J* = 9.6 Hz, 1H), 5.97 (s, 1H), 2.48 (s, 3H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 164.1 (d, *J* =

291.4 Hz), 158.7, 146.9, 132.9 (d, *J* = 8.9 Hz), 130.6, 130.5, 130.1, 129.4, 124.9 (d, *J* = 4.0 Hz), 116.1 (d, *J* = 20.6 Hz), 115.1 (d, *J* = 126 Hz), 69.7, 21.9.

1-Chloro-2-(isocyano(tosyl)methyl)benzene (j). Yellow solid. Yield 69%. mp 103-104 °C. ν_{max}/cm^{-1} 2955, 2923, 2143, 1595, 1441, 1340, 1153, 1085, 813, 715 7.74 (d, *J* = 7.9 Hz, 2H). ^{1}H NMR δ_H (300 MHz; $CDCl_3$) 7.52 (d, *J* = 7.1 Hz, 1H), 7.43-7.32 (m, 5H), 6.23 (s, 1H), 2.47 (s, 3H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 165.8, 147.0, 135.0, 132.2, 131.1, 130.4, 130.2, 130.0, 129.9, 127.6, 125.3, 72.2, 21.9.

1-Bromo-3-(isocyano(tosyl)methyl)benzene (k). Yellow solid. Yield 10%. mp 80-83 °C. ν_{max}/cm^{-1} 2956, 2135, 1592, 1333, 1149, 580. ^{1}H NMR δ_H (300 MHz; $CDCl_3$) 7.61 (d, *J* = 6.9 Hz, 2H), 7.60 (s, 1H), 7.40 (s, 1H), 7.35 (d, *J* = 6.9 Hz, 2H), 7.29 (s, 1H), 7.26 (s, 1H), 5.55 (s, 1H), 2.47 (s, 3H). ^{13}C NMR δ_C (75 MHz; DMSO-d6) 160.9, 147.4, 145.7, 134.1, 132.5, 130.8, 130.3, 130.2, 129.7, 128.0, 122.2, 69.9, 21.9.

2-(*m*-Tolyl)-2-tosylacetonitrile (l). Yellow solid. Yield 65%. mp 126-127 °C. ν_{max}/cm^{-1} 2961, 2136, 1592, 1328, 1151, 580). ^{1}H NMR δ_H (300 MHz; $CDCl_3$) 7.59 (d, *J* = 7.5 Hz, 2H), 7.28 (d, *J* = 7.5, 2H), 7.22-7.11 (m, 4H), 5.62 (s, 1H), 2.41 (s, 3H), 2.29 (s, 3H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 165.9, 164.7, 138.8, 131.6, 130.6, 130.4, 129.9, 129.2, 128.7, 126.6, 125.8, 76.5, 21.9, 21.3.

1-(Isocyano(tosyl)methyl)-2-methoxybenzene (m). Yellow solid. Yield 46%. mp 118-119 °C. ν_{max}/cm^{-1} 2962, 2841, 2143, 1598, 1494, 1463, 1322, 1256, 1159, 818, 751. ^{1}H NMR δ_H (300 MHz; $CDCl_3$) 7.66 (d, *J* = 7.9 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.34-7.29 (m, 3H), 6.99 (t, *J* = 7.3 Hz, 1H), 6.86 (d, *J* = 7.3 Hz, 1H), 6.25 (s, 1H), 3.73 (s, 3H), 2.45 (s, 3H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 164.6, 157.2, 146.3, 132.3, 131.3, 130.5, 129.7, 129.0, 121.0, 115.9, 111.0, 69.9, 55.9, 21.8.

1-(Isocyano(tosyl)methyl)-2-methylbenzene (n). Yellow solid. Yield 82%. mp 118-119 °C. ν_{max}/cm^{-1} 2977, 2137, 1594, 1492, 1463, 1335, 1149, 817, 733. ^{1}H NMR δ_H (300 MHz; $CDCl_3$) 7.68 (d, *J* = 8.9 Hz, 2H), 7.37-7.34 (m, 3H), 7.25-7.19 (m, 3H), 5.89 (s, 1H), 2.47 (s, 3H), 2.43 (s, 3H). ^{13}C NMR δ_C (75 MHz; $CDCl_3$) 165.2, 146.8, 138.0, 131.4, 131.0, 130.7, 130.1, 128.6, 128.0, 126.8, 125.5, 74.9, 22.0, 19.7.

Cellular and enzymatic concentration-response curves.

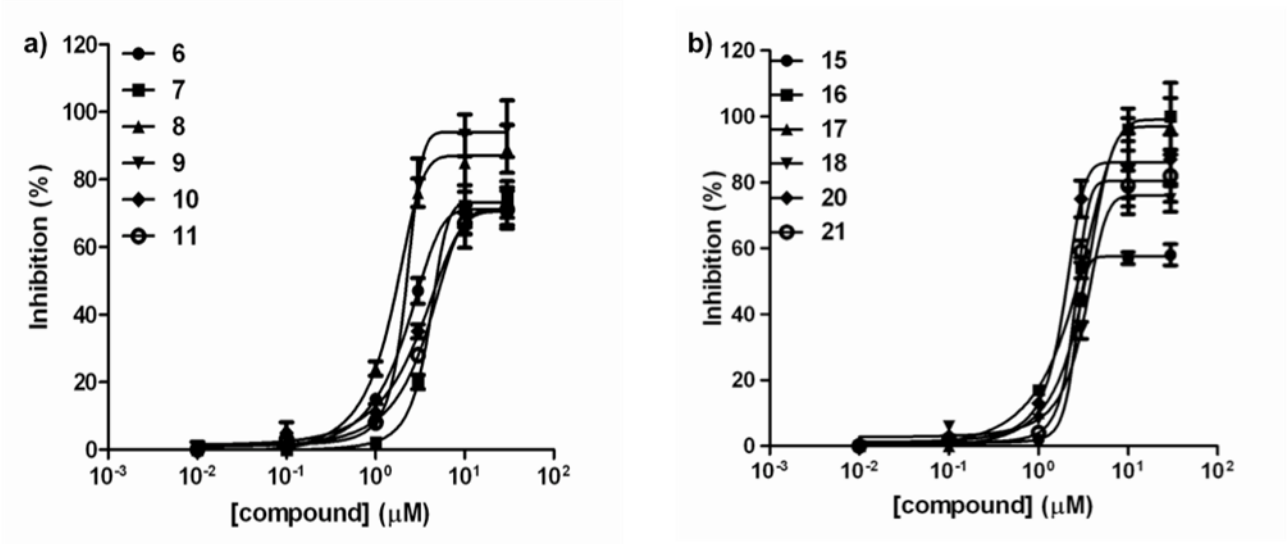


Fig. 2S. Concentration-response curves for tested compounds (a, **6**-**11**; b, **15**-**21**) in IDO1 inhibition cell-based assay. A375 cells were treated (48h) with IFN-γ (500 U/mL) and increasing concentrations (0.01-30 μM) of each compound. The L-KYN levels in cell culture supernatants were measured by HPLC method. The data represent mean ± SEM of at least three independent assays run in triplicate. Percent inhibition against inhibitor concentration was plotted and IC_{50} value was determined. The data were evaluated by GraphPad 5.0.

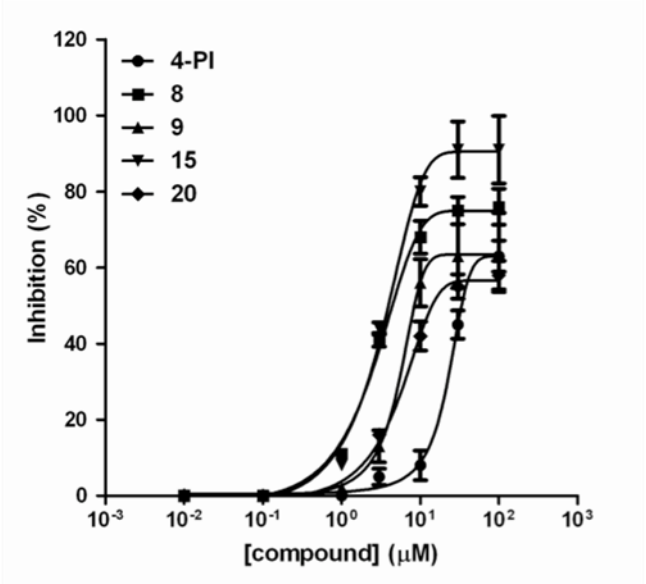


Fig. 3S. Concentration-response curves for **4-PI** and compounds **8**, **9**, **15** and **20** in IDO1 inhibition enzymatic assay. Increasing concentrations (0.01-100 μM) of each inhibitor was added to a standard reaction mixture (see Experimental section) in presence of 50 nM of human recombinant IDO. The reaction was carried out at 37 °C for 60 min. The L-KYN levels were revealed by Ehrlich's reagent and evaluated by a microplate reader. The data represent mean ± SEM of at least three independent assays run in triplicate. Percent inhibition against inhibitor concentration was plotted and IC_{50} value was determined. The data were evaluated by GraphPad 5.0.

Cpd	Hill slope value
1	1.1 ± 0.31
8	1.4 ± 0.53
9	1.9 ± 0.42
15	1.5 ± 0.23
20	1.8 ± 0.37

Table 1S. Hill slope values of the enzymatic curves.

3D-QSAR model generation.

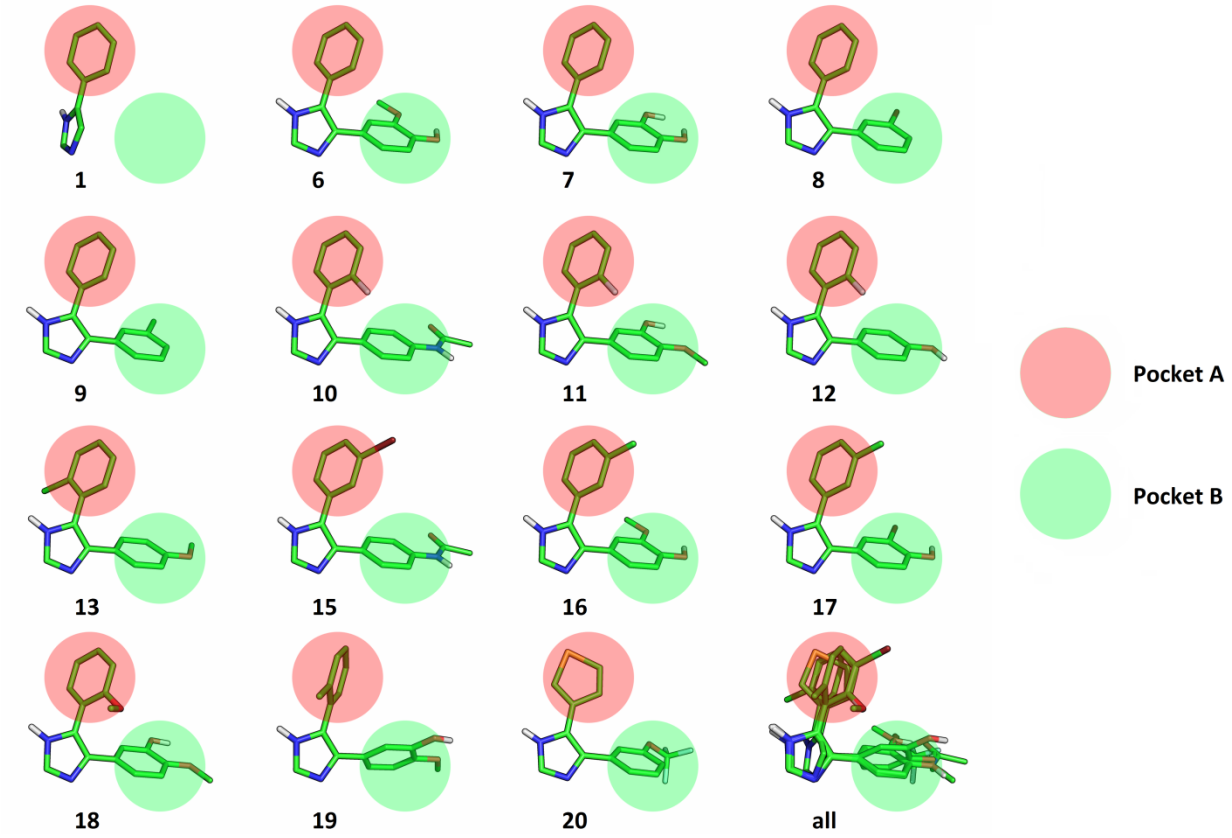


Fig. 4S. Pocket-binding hypotesis of compounds **1** and **6**-**20** based on the 3D-QSAR alignment.

Cpd	Set	Exp IC_{50} µM	Exp pIC_{50}	Pred pIC_{50}
1	Training	150	3.8	3.8
6	Training	3.5	5.5	5.5
7	Training	6.3	5.2	5.2
8	Test	1.5	5.8	5.7
9	Training	1.7	5.8	5.8
10	Training	4.7	5.3	5.4
11	Training	4.9	5.3	5.2
12	Training	124	3.9	3.9
13	Training	150	3.8	3.8
14	-	-	-	-
15	Training	2.9	5.5	5.5
16	Test	3.8	5.4	5.2
17	Training	5.4	5.3	5.3
18	Training	7.2	5.1	5.2
19	Test	56	4.3	4.3
20	Test	2.1	5.7	4.9

Table 2S. Data set.

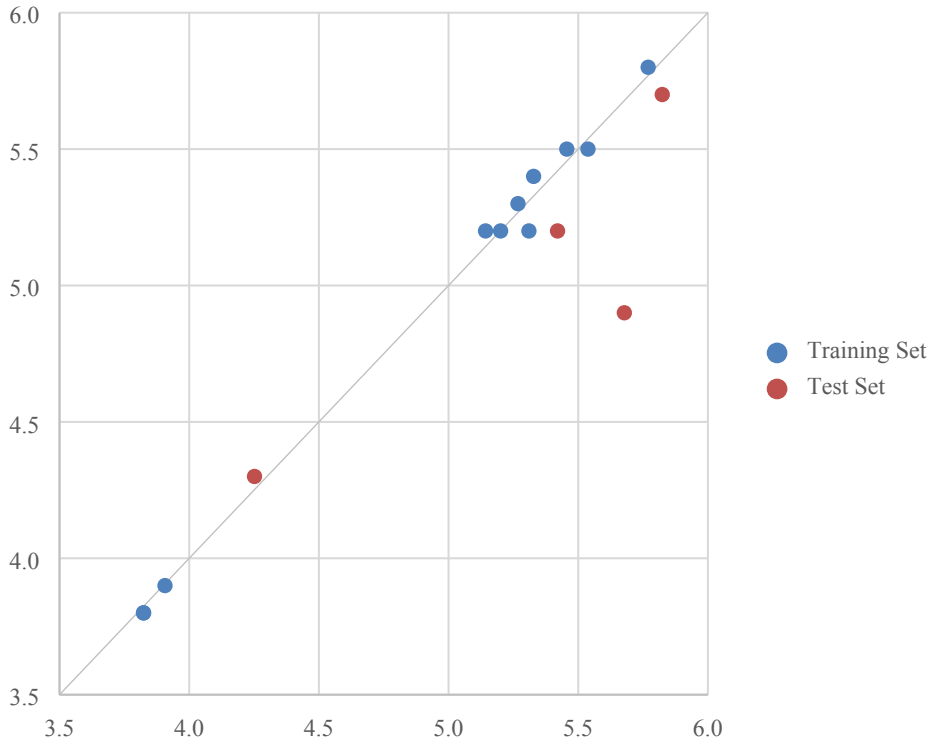


Fig. 5S. Predicted *versus* experimental activity as pIC_{50}.