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Research Article

Design, synthesis, and evaluation of indoleamin-2,3-dioxygenase 1 inhibition activity of novel 5/6-amino indazole derivatives with amide template

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ABSTRACT

Indoleamine 2,3-dioxygenase 1 (IDO1) is an enzyme participating in tryptophan metabolism that has been implicated in numerous cancers. In the present study, a series of novel 5/6-amino indazole derivatives having amide linker were designed, synthesized, and evaluated for IDO1 inhibitory activity. The compounds were initially designed based on the known structural feature of IDO1 active site, and the important role of the indazole scaffold in interaction with IDO1 active site. Thirteen compounds exibited the moderate to excellent inhibitory activity (49% to 100% at the concentration of 1.0 mM). One of them, compound, 2-(6-amino-1*H*-indazol-1-yl)-*N*-(4-chlorophenyl) acetamide (19d), with chloro substituents group at para- position increased the activity upto 100%, equal to that value of the positive control, IDO5L. This research suggests that 5/6-amino indazole moiety combined with amide template is a potential scaffold for IDO1 inhibition as anti-cancer agents.

Keywords:
Aminoindazole, Amide, Indoleamine 2,3-dioxygenase 1, IDO1

1. INTRODUCTION

Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing oxidoreductase which plays the vital role in initiating and catalyzation of tryptophan into kynurenine [1-2]. The accumulation of tryptophan metabolites in the tumor microenvironment suppress effector T cells and potentiate regulatory T cells, thereby promoting tumor cells escape from immune surveillance[3-8]. Several small molecule IDO1 inhibitors are undergoing clinical trials as anticancer agents, such as PF-0684003[9], Novoximod[10], BMS-986205[11] (Figure 1). Studies on IDO1 structure reported that the enzyme's active site was composed of three regions: coenzyme heme, and two hydrophobic pockets: pocket A and pocket B. Pocket A is located above the sixth coordination site of the iron-heme; meanwhile pocket B is located at the binding site entrance[12-13] (Figure 2A).

Our previous study indicated that the indazole scaffold plays an important role in interaction with IDO1 active site residues at pocket A, and the 6-NH group in the 6-aminoindazole template also forms hydrogen bond (HB) with the 7-propionate of the heme ion (Figure 2B)[14].

Based on the well-known structure of IDO1 active site and a crucial role of indazole moiety at the pocket A, we designed a novel scaffold that has an additional pharmacophoric feature target to pocket B (Figure 2C). These new series of compounds contain 5/6-amino indazole template connecting with various substituted-phenyl rings via amide linker, which may interact with the iron ion of hemeglobin the active site of IDO1. In this work, we synthesized newly designed IDO1 inhibitors, determined the structures of novel compounds by IR, MS and NMR spectra. Next, we evaluated the *in vitro* IDO1 inhibition activity of synthesized compounds in order to find the promising IDO1 inhibitors for further investigation on anticancer treatment.

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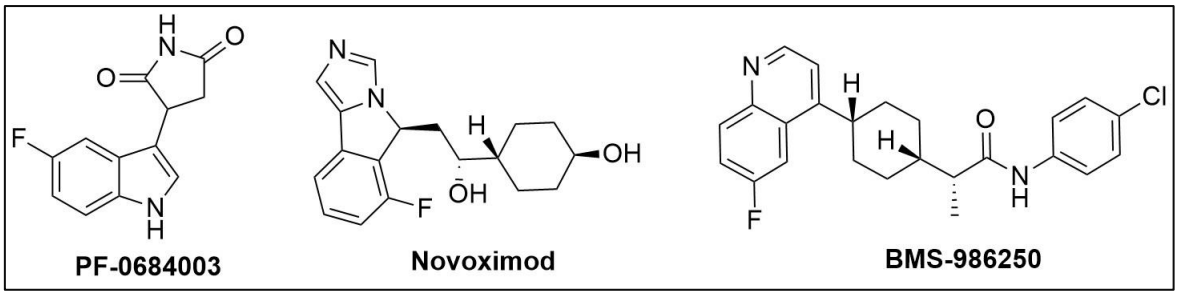


Figure 1. Structure of some known IDO1 inhibitors undergoing clinical trials.

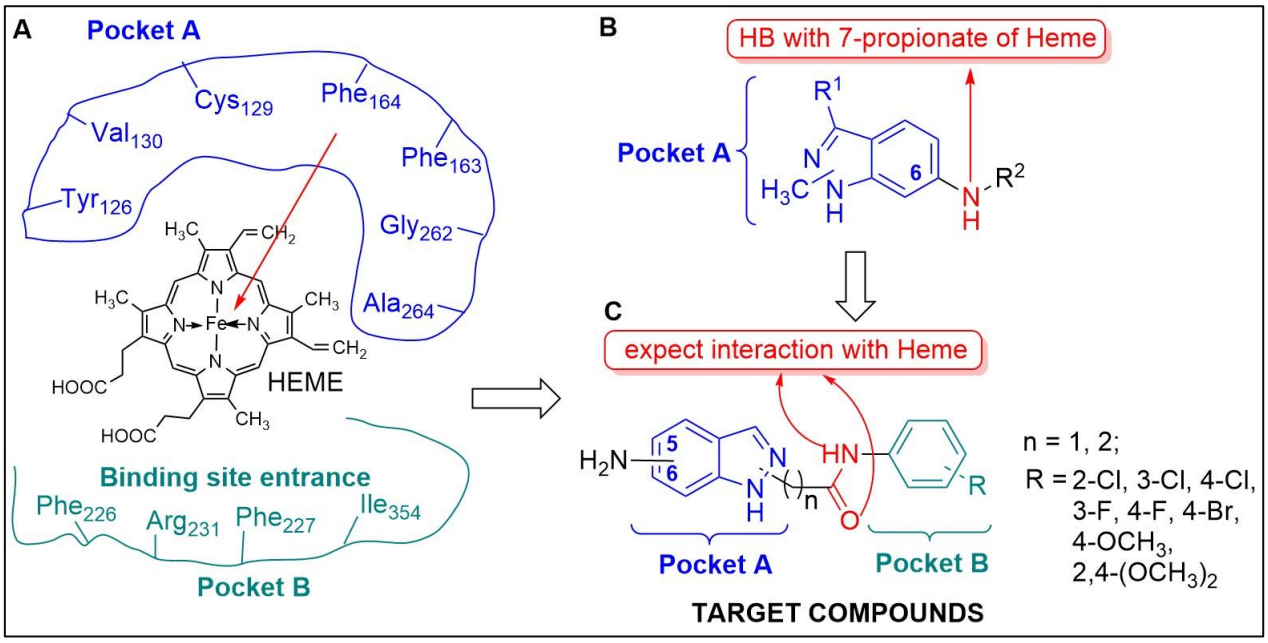


Figure 2. (A) IDO1 active site; (B) Our previous IDO1 inhibitors structures and their interaction in IDO1's active site; (C) The structures of new target compounds.

2. MATERIALS AND METHODS

2.1. Chemistry

Thin layer chromatography using Whatman® 250 µm Silica Gel GF Uniplates and visualized under UV light at 254 nm, was used to check the progress of reactions and preliminary evaluation of compounds' homogeneity. Melting points were measured using a Gallenkamp Melting Point Apparatus (LabMerchant, London, United Kingdom) and were uncorrected. Purification of compounds was carried out using crystallization methods and/or open silica gel column flash chromatography employing Merck silica gel 60 (0.063 to 0.200 mm) as stationary phase. Nuclear magnetic resonance spectra were recorded on a Bruker 500 MHz spectrometer at 500 MHz for ^{1}H-NMR, and 125 MHz for ^{13}C-NMR, with $CDCl_3$ or DMSO-d_6 as solvent unless otherwise indicated. Tetramethylsilane was used as an internal standard. Chemical shifts are reported in parts per million (ppm), downfield from tetramethylsilane. Mass spectra with Electrospray ionization (ESI) mode, were recorded using Mariner® (Azco Biotech, Inc. Oceanside, CA, USA) mass spectrometers. All reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) or Merck unless noted otherwise. Solvents were used directly as purchased unless otherwise indicated.

The synthesis of 5/6-amino indazole template connecting with various substituted-phenyl rings via amide linker (18-22) was carried out as illustrated in Figure 3. Details are described below.

5-Nitroindazole (1) or 6-nitroindazole (2) (326 mg, 2 mmol) was dissolved in acetone (4 mL) or *N*,*N*-dimethylformamide (DMF, 2 mL). K_2CO_3 (55.2 mg, 0.4 mmol) was added. The resulting mixture was heated at 60°C with stirring for 1 hour, then ethyl chloroacetate (0.13 mL, 1.2 mmol) or ethyl 3-chloropropionate (0.17 mL, 1.2 mmol) diluted in 1 mL acetone (or DMF) was dropwise added into the mixture. The reaction mixture was further stirred at 60°C for 4 hours. After the reaction completed, the resulting mixture was quenched with water and extracted with dichloromethane (DCM, 20 mL x 3 times). The organic layer was collected, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain the corresponding nitroindazole ester 3-7 as a yellow solid.

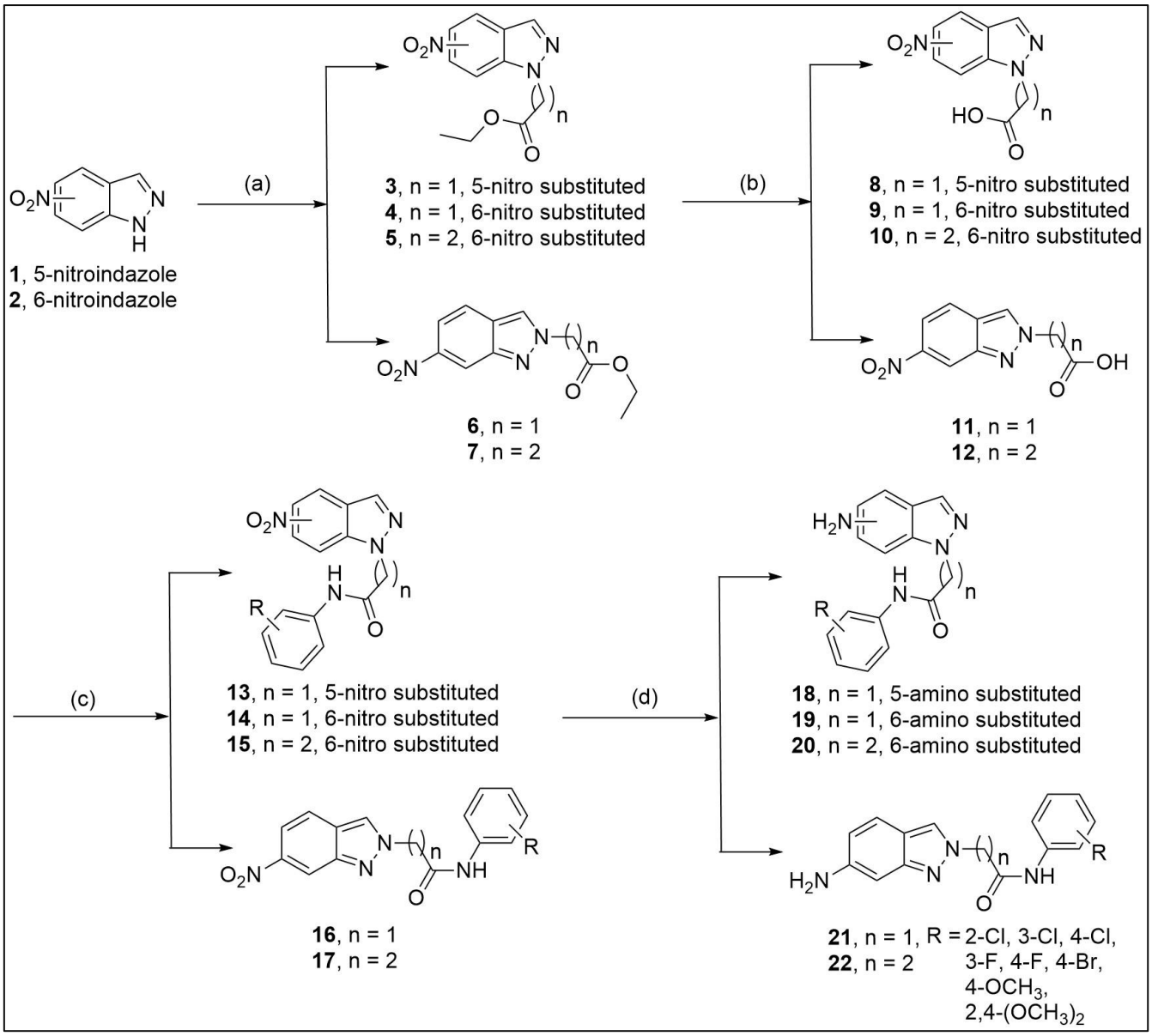


Figure 3. Synthesis of 5/6-amino indazole template connecting with various substituted-phenyl rings via amide linker (**18-22**). Reagents and conditions: (a) ethyl chloroacetate or ethyl 3-chloropropionate, K_2CO_3, acetone or DMF, 60°C, 5h; (b) NaOH, MeOH, r.t, 4 h; (c) Ar-NH_2, EDC.HCl, HOBt, TEA, DCM, r.t, 12 h; (d) $SnCl_2.2H_2O$/HCl, EtOAc, 60°C, 3 h.

The ester 7-11 (1 mmol) was dissolved in 5 mL MeOH. The solution of sodium hydroxide (NaOH, 200 mg, 5 mmol) in MeOH (2 mL) was added slowly to the respective solution of ester. The mixture was stirred at room temperature for 4 hours, then acidified to pH of 2.0-3.0 by concentrated hydrochloric acid, extracted with DCM (20 mL x 3 times). The organic layer was collected, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give respectively acid 8-12 as light yellow solid.

A mixture of acid 8-12 (0.5 mmol) in DCM (5 mL), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrocloride (EDC.HCl, 77.6 mg, 0.5 mmol), 1-hydroxy benzotriazole (HOBt, 65.6 mg, 0.5 mmol), and triethylamine (TEA, 0.14 mL, 1 mmol), aniline derivatives or 2,3-dihydrobenzo[*b*][1,4]dioxin-6-amine (0.6 mmol) was stirred for 12 hours at room temperature. The reaction mixture was diluted with water and extracted with DCM three times. The combines organic extracts were washed with 1N HCl and brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc:*n*-hexane = 1:1 as eluent to obtain amide derivatives 13-17.

$SnCl_2.2H_2O$ (1.1 g, 5 mmol) and 3 drops of concentrated HCl was added to a solution of amide 13-17 (1 mmol) in EtOAc (10 mL), respectively. The reaction mixture was stirred at 60°C until starting material consumed, then diluted with EtOAc, basified by saturated solution of Na_2CO_3, filtered through Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, filtered, concentrated in vacuo, and recrystallized in DCM or purified by flash column chromatography (DCM/MeOH) to obtain the desired products (18-22).

2.2. IDO1 inhibition assay

IDO1 activity assay was performed using IDO1 acitivity assay kit (Biovision), following the instruction of the manufacturer. Briefly, IDO1 was incubated with L-tryptophan in the presence of 1.0 mM of designed compounds or positive control IDO5L (4-amino-*N*-(3-chloro-4-fluorophenyl)-N′-hydroxy-1,2,5-oxadiazole-3-carboximidamide, one of the highest potent IDO1 inhibitors (IC_{50}= 19 nM, HeLa cell assay))[15] at 37°C in 45 min. The Fluorogenic Developer Solution was then added to the reactions to generate fluorescent signals at 37°C in 3hrs. After that, the fluorescent signals were recorded at Ex/Em=402/488 nm. The relative inhibition activity was calculated as the following equation: % Relative Inhibition = (Fsc-Ftc)/Fsc × 100%. All the experiments were repeated independently at least 3 times.

3. RESULTS

3.1. Chemistry

The designed compounds 18-22 were synthesized following scheme 1, their structures were determined by IR, HR-MS, NMR spectra. The detail information of their spectra was given below.

*3.1.1. 2-(5-amino-1H-indazol-1-yl)-N-phenylacetamide (**18a**)*

Off-white solid, yield: 60%. mp: 186.4-187.5°C, $\boldsymbol{R_f}$ = 0.55 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3410, 3323, 3269 (NH amide, NH_2 amine), 3059 (CH aromatic), 1666 (C=O); 1632 (C=N); 1597, 1498 (C=C aromatic). ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ 7.97 (s, 1H, H3), 7.85 (s, 1H, CO-NH), 7.38-7.37 (d, J = 8.0 Hz, 2H, H2', H6'), 7.29-7.25 (m, 3H, H3', H5', H7), 7.09-7.06 (t, J = 7.5 Hz, 1H, H4'), 6.97-6.96 (d, J = 1.5 Hz, 1H, H4), 6.94-6.92 (dd, J_1 = 2.0 Hz, J_2 = 9.0 Hz, 1H, H6), 5.10 (s, 2H, CH_2), 3.68 (s, 2H, NH_2). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 165.78, 141.15, 136.88, 135.78, 134.13, 129.03, 125.31, 124.79, 119.96, 119.69, 109.64, 103.47, 52.71. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{15}N_4O^+$ ($[M+H]^+$) = 267.1240, found 267.1246.

*3.1.2. 2-(5-amino-1H-indazol-1-yl)-N-(2-chlorophenyl) acetamide (**18b**)*

Off-white solid, yield: 70%. mp: 194.5-195.8°C, $\boldsymbol{R_f}$ = 0.52 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, $cm^{-1)}$: 3419, 3346, 3217 (NH amide, NH_2 amine), 3116 (CH aromatic), 1674 (C=O), 1589 (C=N), 1537, 1442 (C=C aromatic), 1055 (C-Cl aromatic). ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ 8.35-8.33 (m, 1H, H6'), 8.23 (s, 1H, CO-NH), 8.00 (s, 1H, H3), 7.28-7.21 (m, 3H, H7, H3', H5'): 7.01-6.97 (m, 2H, H4, H4'), 6.94-6.92 (dd, J_1 = 2.0 Hz, J_2 = 9.0 Hz, H6), 5,15 (s, 2H, CH_2), 3,68 (s, 2H, NH_2). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 166.08, 141.24, 135.75, 134.37, 134.00, 129.02, 127.57, 125.50, 125.03, 122.97, 121.36, 119.53, 109.61, 103.65, 52.86. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}{}^{35}ClN_4O^+$ ($[M+H]^+$) = 301.0851, found 301.0857.

*3.1.3. 2-(5-amino-1H-indazol-1-yl)-N-(4-bromophenyl) acetamide (**18g**)*

Off-white solid, yield: 68%. mp: 203.4-204.8°C, $\boldsymbol{R_f}$ = 0.60 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3408, 3323, 3248 (NH amide, NH_2 amine), 3053 (CH aromatic), 1664 (C=O), 1591 (C=N), 1537, 1487 (C=C aromatic),. ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ 7.97 (s, 1H, H3), 7.38-7.37 (d, J = 8.8 Hz, 2H, H3', H5'), 7.30-7.26 (m, 3H, H2', H6', H7), 6.97-6.93 (m, 2H, H4, H6), 5.08 (s, 2H, CH_2). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 165.97, 141.35, 136.07, 135.85, 134.29, 131.93, 125.28, 121.62, 119.71, 117.12, 109.67, 103.46, 52.59. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}{}^{79}BrN_4O^+$/$C_{15}H_{14}{}^{81}BrN_4O^+$ ($[M+H]^+$) = 345.0346/ 347.0325, found 345.0349/347.0331.

*3.1.4. 2-(5-amino-1H-indazol-1-yl)-N-(4-methoxyphenyl) acetamide (**18h**)*

Off-white solid, yield: 70%. mp: 200.2-201.8°C, $\boldsymbol{R_f}$ = 0.5 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3415, 3259 (NH amide, NH_2 amine), 3057 (CH aromatic), 1664 (C=O), 1604 (C=N), 1548, 1458 (C=C aromatic). ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ 7.96 (s, 1H, H3), 7.70 (s, 1H, CO-NH), 7.61 (m, 3H, H7, H2', H6'), 6.97-6.96 (d, J = 2.0 Hz, 1H, H4), 6,.94-6.92 (dd, J_1 = 2.5 Hz, J_2 = 9.0 Hz, 1H, H6), 6.81 (d, J = 9.0 Hz, 1H, H3', H5'), 5.09 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 165.69, 156.77, 141.25, 135.82, 134.11, 129.95, 125.29, 122.03, 119.59, 114.10, 109.73, 103.46, 55.46, 52.59. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{17}N_4O_2^+$ ($[M+H]^+$) = 297.1346, found 297.1356.

*3.1.5. 2-(5-amino-1H-indazol-1-yl)-N-(2,4-dimethoxy-phenyl)acetamide (**18i**)*

Off-white solid, yield: 70%. mp: 201.4-202.87°C, $\boldsymbol{R_f}$ = 0.68 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3421, 3294 (NH amide, NH_2 amine), 3032 (CH aromatic), 1664 (C=O), 1616 (C=N), 1537, 1504, 1460 (C=C aromatic). ^{1}***H-NMR*** (500 MHz, DMSO-d_6, ppm): δ 9.11 (s, 1H, CO-NH), 7.79 (d, J = 4.5 Hz, 1H, H7), 7.77 (1H, H3), 7.37 (d, J = 9.0 Hz, 1H, H6'), 6.83 (d, J = 2.0 Hz, 1H, H6), 6.81 (s, 1H, H4), 6.76 (s, 1H, H3'), 6,61 (d, J = 2.0 Hz, 1H, H5'), 5.21 (s, 2H, CH_2), 4.85 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3). ^{13}***C-NMR*** (125 MHz, DMSO-d_6, ppm): δ 166.25, 157.14, 151.15, 143.26, 135.39, 132.01, 125.29, 122.82, 120.47, 118.64, 110.57, 104.54, 100.99, 99.31, 56.26, 55.76, 52.17. ***MS*** *(ESI) m/z* calculated for $C_{17}H_{19}N_4O_3^+$ ($[M+H]^+$) = 327.14, found 327.0.

*3.1.6. 2-(6-amino-1H-indazol-1-yl)-N-phenylacetamide (**19a**)*

Off-white solid, yield: 62%. mp: 188.3-189.6°C. R_f = 0.55 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3337 (NH amide), 2930 (CH aromatic), 1670 (C=O), 1283 (C-N), ***^{1}H-NMR*** (500 MHz, DMSO-d_6, ppm): δ10.3 (s, 1H, CO-NH), 7,68 (s, 1H, H3), 7.52 (d, J = 8.0 Hz, 2H, H2', H6'), 7.30 (d, J = 8.5 Hz, 1H, H4), 7.24 (t, J = 8.0 Hz, 2H, H3', H5'), 6.99 (t, J = 8.0 Hz, 1H, H4'), 6,45 (dd, J_1 = 9.0 Hz, J_1 = 2.0 Hz, 1H, H5), 6.40 (s, 1H, H7), 5.24 (s, 2H, NH_2), 4.99 (s, 2H, CH_2). ***^{13}C-NMR*** (125 MHz, DMSO-d_6, ppm): δ166.40, 148.52, 142.84, 139.24, 133.53, 129.30, 123.96, 121.40, 119.62, 116.61, 112.85, 90.50, 52.03. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{15}N_4O^+$ ($[M+H]^+$) = 267.1240, found 267.1246.

*3.1.7. 2-(6-amino-1H-indazol-1-yl)-N-(2-chlorophenyl) acetamide (**19b**)*

Off-white solid, yield: 62%. mp: 195.5-196.9°C. R_f = 0.50 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3373 (NH amide), 2922 (CH aromatic), 1678 (C=O), 1275 (C-N). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.27 (d, J = 7.0 Hz, 1H, H6'), 8.22 (s, 1H, CO-NH), 7.94 (s, 1H, H3), 7.47 (d, J = 8.5 Hz, 1H, H4), 7.14-7.19 (m, 2H, H5', H3'), 6.93 (t, J = 7.5 Hz, H4'), 6.55 (dd, J_1 = 7.5 Hz, J_2 = 1.5 Hz, 1H, H5), 6.49 (s, 1H, H7), 5.07 (s, 2H, CH_2), 3.89 (s, 2H, NH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 165.22, 145.88, 141.10, 134.92, 133.04, 128.00, 126.53, 123.97, 121.96, 121.35, 120.32, 117.06, 112.41, 90.30, 51.75. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}ClN_4O^+$ ($[M+H]^+$) = 301.0851, found 301.0857.

*3.1.8. 2-(6-amino-1H-indazol-1-yl)-N-(3-chlorophenyl) acetamide (**19c**)*

Off-white solid, yield: 71%. mp: 194.5 – 196.2°C, R_f = 0.51 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3358 (NH amide), 3053 (CH aromatic, CH_2), 1664 (C=O), 1290 (C-N),. ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.08 (s, 1H, CO-NH), 7.99 (s, 1H, H3), 7.53 (d, J = 8.5 Hz, 1H, H4), 7.52 (s, 1H, H2'), 7.26 (d, J = 6.0 Hz, 1H, H6'), 7.19 (t, J = 8.0 Hz, 1H, H5'), 7.06 (d, J = 8.0 Hz, 1H, H4'), 6.64 (dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz, 1H, H5), 6.56 (s, 1H, H7), 5.01 (s, 2H, CH_2), 3.98 (s, 2H, NH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ166.09, 147.07, 142.26, 138.17, 135.95, 134.60, 129.93, 124.77, 122.38, 120.10, 118.02, 117.83, 113.57, 91.19, 52.33. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}ClN_4O^+$ ($[M+H]^+$) = 301.0851, found 301.0859.

*3.1.9. 2-(6-amino-1H-indazol-1-yl)-N-(4-chlorophenyl) acetamide (**19d**)*

Off-white solid, yield: 69%. mp: 195.5-196.9°C. R_f = 0.50 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3348 (NH amide), 3061 (CH aromatic, CH_2), 1673 (C=O), 1277 (C-N),. ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.04 (s, 1H, CO-NH), 7.99 (s, 1H, H3), 7.53 (d, J = 9.0 Hz, 1H, H4), 7.34-7.37 (m, 2H, H2', H6'), 7.22 - 7.26 (m, 2H, H3', H5'), 6.63 (dd, J_1 = 8.5 Hz, J_2 = 2,0 Hz, 1H, H5), 6.56 (s, 1H, H7), 5.01 (s, 2H, CH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ166.02, 147.05, 142.26, 135.91, 135.62, 129.73, 128.97, 121.29, 121.29, 117.82, 113.55, 91.20, 52.30. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}ClN_4O^+$ ($[M+H]^+$) = 301.0851, found 301.0861.

*3.1.10. 2-(6-amino-1H-indazol-1-yl)-N-(3-fluororophenyl) acetamide (**19e**)*

Off-white solid, yield: 72%. mp: 188.3-189.7°C. R_f = 0.48 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3280 (NH amide), 3116 (CH aromatic, CH_2), 1685 (C=O), 1279 (C-N), 1219 (C-F). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.14 (s, 1H, CO-NH), 7.99 (s, 1H, H3), 7.53 (d, J = 8.5 Hz, 1H, H4), 7.26 (s, 1H, H2'), 7.18-7.22 (m, 1H, H5'), 7.02 (d, J = 8,0 Hz, 1H, H6'), 6.77-7.80 (m, 1H, H4'), 6.63 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H, H5), 6.57 (s, 1H, H7), 4.59 (s, 2H, CH_2), 3.98 (s, 2H, NH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 166.09, 163.86-161.91 ($^1J_{C-F}$ = 243.63 Hz), 147.08, 142.26, 138.58-138.49 ($^3J_{C-F}$ = 10.63 Hz), 135.90, 130.05, 129.97 ($^3J_{C-F}$ = 9.38 Hz), 122.36, 117.82, 115.31-115.29 ($^4J_{C-F}$ = 2.88 Hz), 113.57, 111.52-111.35 ($^2J_{C-F}$ = 21.23 Hz), 107.62-107.41 (^2JC-F = 26.13 Hz), 91.23, 52.,37. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}ClN_4O^+$ ($[M+H]^+$) = 285.1152, found 285.1153.

*3.1.11. 2-(6-amino-1H-indazol-1-yl)-N-(4-fluorophenyl) acetamide (**19f**)*

Off-white solid, yield: 68%. mp: 186.1-187.8°C. R_f = 0.46 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3261 (NH amide), 3059 (CH aromatic, CH_2), 1666 (C=O), 1281 (C-N), 1215 (C-F). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ 7.99 (s, 1H, H3), 7.95 (s, 1H, CO-NH), 7.54 (d, J = 8.5 Hz, 1H, H4), 7.35-7.38 (m, 2H, H2', H6'), 6.95-6.98 (m, 2H, H3', H5'). 6,63 (dd, J_1 = *7.5* Hz, J_2 = 2.0 Hz, 1H, H5), 6.57 (s, 1H, H7), 5.02 (s, 2H, CH_2), 3.97 (s, 2H, NH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ165.99, 160.51-158.64 ($^1J_{C-F}$ = 242.63 Hz), 147.02, 142.24, 135.86, 133.02, 132.99 ($^4J_{C-F}$ = 2.88 Hz), 122.35, 121.98-121.92 ($^3J_{C-F}$ = 10.13 Hz), 117.84, 115.53 ($^2J_{C-F}$ = 22.38 Hz), 113.53, 91.23, 52.27. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}{}^{35}ClN_4O^+$ ($[M+H]^+$) = 285.1152, found 285.1153.

*3.1.12. 2-(6-amino-1H-indazol-1-yl)-N-(4-bromophenyl) acetamide (**19g**)*

Off-white solid, yield: 66%. mp: 203.1-204.7°C. R_f = 0.52 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3369 (NH amide), 2934 (CH aromatic), 1670 (C=O), 1278 (C-N). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.03 (s, 1H, CO-

NH), 7.99 (s, 1H, H3), 7.54 (d, *J* = 8.5 Hz, 1H, H4), 7.38 (d, *J* = 9.0 Hz, 2H, H2', H6'), 7.31 (d, *J* = 9.0 Hz, 2H, H3', H5'), 6.63 (d, *J* = 8.0 Hz, 1H, H5), 6.55 (s, 1H, H7), 5.00 (s, 2H, CH_2), 3.97 (s, 2H, NH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ166.02, 147.05, 142.27, 136.13, 135.94, 131.93, 122.37, 121.60, 117.83, 117.35, 113.55, 91.91, 52.31. ***HRMS*** *(ESI) m/z* calculated for $C_{15}H_{14}{}^{79}BrN_4O^+$/ $C_{15}H_{14}{}^{81}BrN_4O^+$ ($[M+H]^+$) = 345.0346/347.0325, found 345.0349/347.0328.

*3.1.13. 2-(6-amino-1H-indazol-1-yl)-N-(4-methoxyphenyl) acetamide (**19h**)*

Off-white solid, yield: 61%. mp: 199.2-201.0°C. R_f = 0,40 (EtOAc:*n*-hexane = 1:1). ***^{1}H-NMR*** (500 MHz, $CDCl_3$ + CD_3OD, ppm): δ8.32 (s, 1H, CO-NH), 7.84 (s, 1H, H3), 7.41 (d, *J* = 10.0 Hz, 1H, H4), 7.25 (d, *J* = 6.80 Hz, 2H, H2', H6'), 6.72 (d, *J* = 9.0 Hz, 2H, H3', H5'), 6.52-6.54 (m, 2H, H5, H7), 4.90 (s, 2H, CH_2), 3.67 (s, 3H, OCH_3), 3.01 (s, 2H, NH_2). ^{13}C-NMR (125 MHz, $CDCl_3$ + CD_3OD, ppm): δ166.02, 156.52, 147.07, 142.14, 135.09, 129.99, 121.93, 121.82, 117.49, 113.91, 113.90, 91.34, 55.26, 52.02. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{17}N_4O_2^+$ ($[M+H]^+$) = 297.1346, found 297.1364.

*3.1.14. 2-(6-amino-1H-indazol-1-yl)-N-(2,4-dimethoxy-phenyl)acetamide (**19i**)*

Off-white solid, yield: 63%. mp: 202.8-204.5°C. R_f = 0.35 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3360 (NH amide), 2920 (CH aromatic), 1670 (C=O), 1276 (C-N). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.15 (d, *J* = 9.0 Hz, H6'), 7.98 (s, 2H, N-H, H3), 7.53 (d, *J* = 8.5 Hz, H4), 6.61 (d, *J* = 9.0 Hz, *J* = 2.0 Hz, H5), 6.56 (s, 1H, H7), 6.42 (dd, *J* = 9.0 Hz, *J* = 2.5 Hz, H5'), 6.35 (d, *J* = 2.5 Hz, 1H, H3'), 5.04 (s, 2H, CH_2), 3.93 (s, 2H, NH_2), 3.75 (s, 3H, 3-OCH_3), 3.62 (s, 3H, 4-OCH_3). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ165.48, 156.74, 149.45, 146.71, 142.08, 135.34, 122.13, 120.58, 118.06, 113.26, 103.72, 98.67, 91.60, 55.71, 55.51, 52.71. ***HRMS*** *(ESI) m/z* calculated for $C_{17}H_{19}N_4O_3^+$ ($[M+H]^+$) = 327.1452, found 327.1456.

*3.1.15. 3-(6-amino-1H-indazol-1-yl)-N-phenylpropana-mide (**20a**)*

Off-white solid, yield: 82%. mp: 195.8 - 197.5°C, R_f = 0.29 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3377 (NH amide), 2920 (CH aromatic, CH_2), 1678 (C=O). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.10 (s, 1H, CO-NH), 7.87 (s, 1H, H3), 7.46 (d, *J* = 8.5 Hz, 1H, H4), 7.39 (d, *J* = 8.0 Hz, 2H, H2', H6'), 7.26 (t, *J* = 8.0 Hz, 2H, H3', H5'), 7.06 (t, *J* = 7.5 Hz, 1H, H4'), 6.59 (s, 1H, H7), 6.56 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H, H5), 4.59 (t, *J* = 6.0 Hz, 2H, N-CH_2), 2.99 (t, *J* = 6.0 Hz, 2H, CH_2-CO). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ166.00, 146.24, 141.48, 137.80, 133,71, 128.88, 124.31, 121.95, 119.98, 117.80, 112.98, 91.70, 44.36, 37.66, 29.70, ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{17}N_4O^+$ ($[M+H]^+$) = 281.1397, found 281.1401 .

*3.1.16. 3-(6-amino-1H-indazol-1-yl)-N-(2-chlorophenyl) propanamide (**20b**)*

Off-white solid, yield: 76%. mp: 203.8 - 204.9°C. R_f = 0,31 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3278 (NH amide), 2916 (CH aromatic, CH_2), 1657 (C=O). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.24 (d, *J* = 8.0 Hz, 1H, H6'), 7.97 (s, 1H, CO-NH), 7.85 (s, 1H, H3), 7.45 (d, *J* = 8.5 Hz, 1H, H3'), 7.31 (d, *J* = 8.0 Hz, 1H, H3'), 7.22 (t, *J* = 8.0 Hz, 1H, H5'), 7.01 (t, *J* = 7.5 Hz, 1H, H4'), 6.63 (s, 1H, H7), 6.55 (dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz, 1H, H5), 4.61 (t, *J* = 6.5 Hz, 2H, N-CH_2), 3.09 (t, *J* = 6.5 Hz, 2H, CH_2-CO). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 169.07, 146.05, 141.48, 134.44, 133.90, 129.03, 127.55, 124.81, 123.06, 121.98, 121.93, 117.92, 112.80, 91.80, 44.20, 31.94. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{17}{}^{35}ClN_4O^+$ ($[M+H]^+$) = 315.1007, found 315.1014.

*3.1.17. 3-(6-amino-1H-indazol-1-yl)-N-(4-bromophenyl) propanamide (**20g**)*

Off-white solid; yield: 75%. mp: 206.3 - 207.9°C. R_f = 0.32 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3341 (NH amide), 2941 (CH aromatic, CH_2), 1668 (C=O). ***^{1}H-NMR*** (500 MHz, DMSO-d_6, ppm): δ 10.05 (s, 1H, CO-NH), 7.63 (s, 1H, H3), 7.44 (d, *J* = 8.5 Hz, 2H, H3', H5'), 7.37 (d, *J* = 9.0 Hz, 2H, H2', H6'), 7.25 (d, *J* = 8.5 Hz, 1H, H4), 6.46 (s, 1H, H7), 6.41 (d, *J* = 8.5 Hz, 1H, H5), 5.24 (s, 2H, NH_2), 4.35 (t, *J* = 7.0 Hz, 2H, N-CH_2), 2.80 (t, *J* = 7.0 Hz, 2H, CH_2-CO). ***^{13}C-NMR*** (125 MHz, DMSO-d_6, ppm): δ 169.50, 148.33, 141.79, 138.89, 133.16, 131.96, 121.47, 121.41, 116.49, 115.14, 112.87, 90.30, 90.24, 44.07, 36.73. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{16}{}^{79}BrN_4O^+$/ $C_{16}H_{16}{}^{81}BrN_4O^+$ ($[M+H]^+$) = 359.0502/361.0482, found 359.0504/361.0484.

*3.1.18. 3-(6-amino-1H-indazol-1-yl)-N-(4-methoxyphenyl) propanamide (**20h**)*

Off-white solid, yield: 78%. mp: 197.7 - 198.6°C. R_f = 0.35 (EtOAc:*n*-hexane = 1 : 1). ***IR*** (KBr, cm^{-1}): 3366 (NH amide), 2949 (CH aromatic, CH_2), 1688 (C=O). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ7,98 (s, 1H, CO-NH), 7.85 (s, 1H, H3), 7.45 (d, *J* = 8.5 Hz, 1H, H4), 7.24 (d, *J* = 9.0 Hz, 2H, H3'), 6.78 (d, *J* = 9.0 Hz, 2H, H2'), 6.58 (s, 1H, H7), 6.55 (dd, J_1 = 8.5 Hz, J_2 = 1,5 Hz, 1H, H5), 4.58 (t, *J* = 6.5 Hz, 2H, N-CH_2), 3.75 (s, 3H, OCH_3), 2.94 (t, *J* = 6.5 Hz, 2H, CH_2-CO). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 168.88, 156.46, 146.23, 141.50, 133.69, 130.87, 121.99, 121.90, 117.76, 114.04, 112.96, 91.77, 55.46, 44.46, 37.47. ***HRMS*** *(ESI) m/z* calculated for $C_{17}H_{19}N_4O_2^+$ ($[M+H]^+$) = 311.1503, found 311.1508.

*3.1.19. 3-(6-amino-1H-indazol-1-yl)-N-(2,4-dimethoxy-phenyl)propanamide (**20i**)*

Off-white solid, yield: 75%. mp: 200.3 - 202.1°C; R_f = 0.36 (EtOAc:*n*-hexane = 1 : 1). ***IR*** (KBr, cm^{-1}): 3350 (NH amide), 2918 (CH aromatic, CH_2), 1639 (C=O). ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.13 (d, *J* = 8.5 Hz, 1H, H6'), 7.83 (s, 1H, H3), 7.67 (s, 1H, CO-NH), 7.45 (d, *J* = 8.5 Hz, 1H, H4), 6.66 (s, 1H, H7), 6.54 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H, H5), 6.44 (s, 1H, H3'), 6.41 (d, *J* = 9.0 Hz, 1H, H5'), 4.61 (t, *J* = 7.0 Hz, 2H, N-CH_2), 3.76 (s, 6H, OCH_3), 3.00 (t, *J* = 7.0 Hz, 2H, CH_2-CO). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ168.24, 156.51, 149.34, 145.93, 141.42, 133.65, 121.75, 120.91, 117.89, 112.69, 103.68, 98.56, 92.01, 55.59, 55.53, 44.52, 37.64, 31.94, 29.71, 29.34, 22.67. ***HRMS*** *(ESI) m/z* calculated for $C_{18}H_{20}N_4O_3^+$ ($[M+H]^+$) = 341.1608, found 341.1626.

*3.1.20. 2-(6-amino-2H-indazol-2-yl)-N-phenylacetamide (**21a**)*

Off-white solid, yield: 67%. mp: 186.3-187.8°C. R_f = 0.56 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3439, 3352 (NH amide, NH_2), 2958 (CH aromatic), 1629 (C=O. ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ9.01 (s, 1H, CO-NH), 7.83 (s, 1H, H3), 7.43 (d, *J* = 9.0 Hz, 1H, H4), 7.37 (d, *J* = 7.5 Hz, 2H, H2', H6'), 7.22 (d, *J* = 7.5 Hz, 2H, H3', H5'), 7.03 (t, *J* = 7.5 Hz, 1H, H4'), 6.76 (s, 1H, H7), 6.60 (d, *J* = 2.0 Hz, 1H, H5), 5.04 (s, 2H, CH_2). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ164.43, 145.92, 137.05, 132.47, 130.88, 128.94, 128.81, 125.62, 124.75, 121.38, 117.19, 96.12, 56.57.

*3.1.21. 2-(6-amino-2H-indazol-2-yl)-N-(2,4-dimethoxy-phenyl)acetamide (**21i**)*

Off-white solid, yield: 69%. mp: 204.1-205.6°C. R_f = 0.59 (EtOAc:*n*-hexane = 1:1). ^{1}***H-NMR*** (500 MHz, $CDCl_3$, ppm): δ 8.99 (s, 1H, CO-NH), 8,15 (d, *J* = 9.0 Hz, H6'), 7.89 (s, 1H, H3), 7.70 (d, *J* = 9.0 Hz, 1H, H4), 6.85 (s, 1H, H7), 6.66 (dd, J_1 = 9.0 Hz, J_2 = 1.5 Hz, 1H, H5), 6.42 (d, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H, H5'), 6.37 (d, *J* = 2.5 Hz, 1H, H3'), 5.10 (s, 2H, CH_2), 3.84 (s, 2H, NH_2), 3.75 (s, OCH_3), 3.66 (s, 3H, OCH_3). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ164.09, 156.78, 151.82, 149.62, 145.54, 125.17, 121.31, 120.67, 120.47, 117.19, 117.02, 103.68, 98.66, 96.36, 56.79, 55.81, 55.49. ***MS*** *(ESI) m/z* calculated for $C_{17}H_{19}N_4O_3^+$ ($[M+H]^+$) = 327.14, found 327.0.

*3.1.22. 3-(6-amino-2H-indazol-2-yl)-N-phenylpropana-mide (**22a**)*

Off-white solid, yield: 60%. mp: 190.1-192.3°C, R_f = 0.50 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3421, 3329, 3228 (NH amide, NH_2 amine), 3014 (CH aromatic), 1666 (C=O), 1637 (C=N). ^{1}***H-NMR*** (500 MHz, DMSO-d_6, ppm): δ9.93 (s, 1H, CONH), 7.92 (s, 1H, H3), 7.47 (d, *J* = 8.0 Hz, 2H, H2', H6'), 7.26 (d, *J* = 9.0 Hz, 1H, H4), 7.20 (d, *J* = 7.5 Hz, 2H, H3', H5'), 6.95 (t, *J* = 7.0 Hz, 1H, H4), 6.42 (d, *J* = 9.0 Hz, 1H, H5), 6.37 (s, 1H, H7), 4.92 (s, 2H, NH_2), 4.47 (t, *J* = 6.5 Hz, 2H, CH_2), 2.90 (t, *J* = 6.5 Hz, 2H, CH_2). ^{13}***C-NMR*** (125 MHz, DMSO-d_6, ppm): δ169.00, 150.57, 146.83, 139.47, 129.16, 123.89, 123.65, 120.93, 119.53, 116.35, 115.77, 94.41, 48.56, 37.36. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{17}N_4O^+$ ($[M+H]^+$) = 281.1397, found 281.1405.

*3.1.23. 3-(6-amino-2H-indazol-2-yl)-N-(2-chlorophenyl) propanamide (**22b**)*

Off-white solid, yield: 64%, mp: 195.2-197.0°C, R_f = 0.48 (EtOAc:*n*-hexane = 1:1). ^{1}***H-NMR*** (500 MHz, DMSO-d_6, ppm): δ9.67 (s, 1H, CONH), 8.03 (s, 1H, H3), 7.67 (d, *J* = 7.5 Hz, 1H, H6'), 7.47 (dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H, H3'), 7.35 (d, *J* = 9.0 Hz, 1H, H4), 7.31 (td, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1H, H5'), 7.19 (td, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H, H4'), 6.51 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz,1H, H5), 6.48 (d, *J* = 1.0 Hz, 1H, H7), 4.72 (t, *J* = 6.0 Hz, 2H, CH_2), 3.17 (t, *J* = 6.0 Hz, 2H, CH_2). ^{13}***C-NMR*** (125 MHz, $CDCl_3$, ppm): δ168.61, 150.75, 145.00, 134.38, 129.11, 127.52, 124.96, 124.19, 123.30, 122.15, 121.18, 116.68, 116.31, 96.73, 60.41, 48.78, 38.09. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{17}{}^{35}ClN_4O^+$ ($[M+H]^+$) = 315.1007, found 315.1014.

*3.1.24. 3-(6-amino-2H-indazol-2-yl)-N-(4-bromophenyl) propanamide (**22g**)*

Off-white solid, yield: 71%, mp: 200.3-202.5°C, R_f = 0.55 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3468, 3379, 3246 (NH amide, NH_2 amine), 3047 (CH aromatic), 1680 (C=O), 1635 (C=N), 1598, 1485 (C=C aromatic),. ^{1}***H-NMR*** (500 MHz, DMSO-d_6, ppm): δ 10.17 (s, 1H, CONH), 7.92 (s, 1H, H3), 7.46 (d, *J* = 8.5 Hz, 2H, H2', H6'), 7.38 (m, 2H, H3', H5'), 7.25 (d, *J* = 9.0 Hz, 1H, H7), 6.42 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H, H6'), 6.36 (s, 1H, H8'), 4.92 (s, 2H, NH_2), 4,47 (t, *J* = 6.5 Hz, 2H, CH_2), 2.92 (t, *J* = 6.5 Hz, 2H, CH_2). ^{13}***C-NMR*** (125 MHz, DMSO-d_6, ppm): δ169.25, 150.57, 146.84, 138.86, 131.97, 123.92, 121.46, 120.93, 116.37, 115.76, 115.17, 94.40, 48.46, 37.36. ***HRMS*** *(ESI) m/z* calculated for $C_{16}H_{16}{}^{79}BrN_4O^+$/ $C_{16}H_{16}{}^{81}BrN_4O^+$ ($[M+H]^+$) = 359.0502/361.0482, found 359.0515/361.0493.

*3.1.25. 3-(6-amino-2H-indazol-2-yl)-N-(4-methoxyphenyl) propanamide (**22h**)*

Off-white solid, yield: 74%, mp: 194.2-196.3°C, R_f = 0.59 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3442, 3358, 3296 (NH amide, NH_2 amine), 3062 (CH aromatic) 1639 (C=O), 1614 (C=N), 1602, 1487 (C=C aromatic). ^{1}***H-NMR*** (500 MHz, DMSO-d_6, ppm): δ 9.78 (s, 1H,

CONH), 7.91 (s, 1H, H3), 7.37 (d, *J* = 9.0 Hz, 2H, H2', H6'), 7.25 (d, *J* = 9.0 Hz, 1H, H4), 6.77 (d, *J* = 8.5 Hz, 2H, H3', H5'), 6.42-6.40 (d, *J* = 10.0 Hz, 1H, H5), 6.37 (s, 1H, H7), 4.93 (s, 2H, NH_2), 4.45 (t, *J* = 6.5 Hz, 2H, CH_2), 3.61 (s, 3H, OCH_3), 2.86 (t, *J* = 6.5 Hz, 2H, CH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ 168.44, 155.62, 150.55, 146.81, 132.65, 123.86, 121.08, 120.93, 116.34, 115.78, 114.29, 94.43, 79.71, 55.61, 48.67, 37.26. ***HRMS (ESI) m/z*** calculated for $C_{17}H_{19}N_4O_2^+$ ($[M+H]^+$) = 311.1503, found 311.1511.

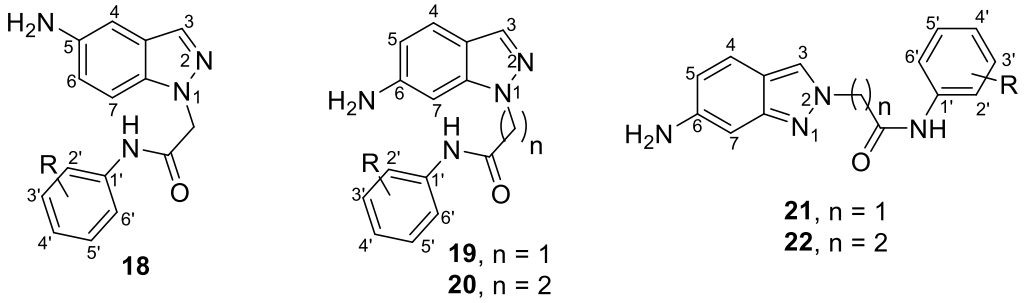
*3.1.26. 3-(6-amino-2H-indazol-2-yl)-N-(2,4-dimethoxyphenyl)propenamide (**22i**)*

Off-white solid, yield: 73%, mp: 199.2-201.4°C, R_f = 0,49 (EtOAc:*n*-hexane = 1:1). ***IR*** (KBr, cm^{-1}): 3442, 3358, 3296 (NH amide, NH_2 amine), 3012 (CH aromatic) 1639 (C=O), 1614 (C=N), 1533, 1456 (C=C aromatic). ***^{1}H-NMR*** (500 MHz, $CDCl_3$, ppm): δ8.13 (d, *J* = 9.0 Hz, 1H, H6'), 7.83 (s, 1H, H3), 7.60 (s, 1H, CONH), 7.42 (d, *J* = 9.0 Hz, 1H, H4), 6.79 (s, 1H, H7), 6.57 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H, H5), 6.43 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H, H5'), 6.39 (d, *J* = 2.5 Hz, 1H, H3'), 4.71 (t, *J* = 6.5 Hz, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.08 (t, *J* = 6.5 Hz, 2H, CH_2). ***^{13}C-NMR*** (125 MHz, $CDCl_3$, ppm): δ167.69, 156.58, 150.72, 149.35, 144.82, 124.19, 121.27, 120.87, 120.82, 116.69, 116.07, 103.64, 98.561, 96.79, 55.53, 49.13, 38.23, 29.70. ***HRMS (ESI) m/z*** calculated for $C_{18}H_{20}N_4O_3^+$ ($[M+H]^+$) = 341.1608, found 341.1618.

3.2. Bioactivity

The series of compounds 18-22 were evaluated for their IDO1 inhibition activities at 1.0 mM. IDO5L was used as a positive control. The results are presented in the Table 1.

Table 1. IDO1 inhibition of synthesized compounds 18-22 at 1.0 mM.



Cpd	R	IDO1 inhibition (%)	Cpd	R	IDO1 inhibition (%)
18a	H	22	**20a**	H	48
18b	2-Cl	67	**20b**	2-Cl	38
18g	4-Br	76	**20g**	4-Br	82
18h	4-OCH_3	N/E[1]	**20h**	4-OCH_3	64
18i	2,4-$(OCH_3)_2$	N/E	**20i**	2,4-$(OCH_3)_2$	35
19a	H	87	**21a**	H	39
19b	2-Cl	98	**21i**	2,4-$(OCH_3)_2$	N/A[2]
19c	3-Cl	49	**22a**	H	N/A
19d	4-Cl	100	**22b**	2-Cl	N/E
19e	3-F	57	**22g**	4-Br	41
19f	4-F	65	**22h**	4-OCH_3	N/A
19g	4-Br	99	**22i**	2,4-$(OCH_3)_2$	15
19h	4-OCH_3	79	**IDO5L**		100
19i	2,4-$(OCH_3)_2$	95	**Negative control (solvent)**		0

[1]N/E: No effect; [2]N/A: Not available (the compound was precipitated in the reaction buffer)

4. DISCUSSION

4.1. Chemistry

The designed compounds were synthesized through four steps as described in Scheme 1. The first reaction was alkylation of 5- or 6-nitro-1*H*-indazole with ethyl chloroacetate or ethyl 3-chloropropionate under basic conditions (K_2CO_3). KI was used as catalystic agent to activate and increase the reaction rate to obtain ester 3-7. The N^1-alkylindazoles (3-5) were major products, meanwhile N^2-alkylindazoles (6-7) were minor products. The structures of two isomers were confirmed by 2D-NMR before carrying out a hydrolysis reaction used sodium hydroxide in MeOH to afford corresponding acids 8-12. Next, these acids were coupled with aniline derivatives under EDC amide coupling condition or one-pot amide coupling synthesis using thionyl chloride to obtain amide

derivatives 13-17. The final amine derivatives 18-22 were prepared by reduction reaction using $SnCl_2.2H_2O$ in acidic condition.

The structures of synthesized compounds were confirmed by IR, MS, and NMR spectra. The IR spectral data was consistent with the expected structural formula of 18-22 with the appearance of valence fluctuations of the NH amide, -NH_2, C=O, C-N, CH aromatic, CH_2, etc. The ^{1}H-NMR spectrum of the all compounds showed a single peak with a chemical shift of about 8 ppm (in $CDCl_3$), or about 9.3 ppm (in DMSO) corresponding to the proton of NH amide. The proton of the amine group (-NH_2), methylene group, ethylene group were also represented in ^{1}H-NMR spectrum. The ^{13}C-NMR data showed full carbon number of synthesized compounds 18-22. The mass spectra indicated the ion molecular peak ([M+H]$^+$) which was matched with the molecular weight of target compounds.

4.2. Biological activity

The synthesized amide derivatives 18-22 were evaluated for the *in vitro* IDO1 inhibition activities at 1.0 mM using fluorescence method followed the instruction of BioVision's IDO1 Inhibitor Screening Kit. **IDO5L** was used as positive control. These results in Table 1 demonstrated that 20 compounds had IDO1 inhibitory activity. Among them, 13 compounds had moderate to excellent activity ranging from 49% to 100%. The N^1-alkylindazoles compounds **19**, **20** presented better activities than corresponding N^2-alkylindazoles compounds **21**, **22**. Among compounds with the same substituent groups in benzene ring, the 6-substituted-1*H*-indazole derivatives (**19a, b, g, h, i**) showed higher inhibition percentages than the 5-substituted-1*H*-indazole derivatives (**18a, b, g, h, i**). Compounds with acetamide linkers between indazole moiety and benzene ring (**19a, b, g, h, i**) had more potential IDO1 inhibitory activity than those contained propanamide linkers (**20a, b, g, h, i**). Considering the influence of substituents on the benzene ring, the activities of the derivatives were different regarding to different substituents as well as substituent positions on the benzene ring. Halogen (-F, -Cl, Br) subsituents indicated better IDO1 inhibitory activity than –OCH_3, or –$(OCH_2)_3$ substituted compounds. Moreover, with the same substituted group, compounds with 4-substituted groups showed more potent activites than compounds with 2- or 3- substituted. As demonstrated in Table 1, compounds **19d** (4-Cl substituted), **19f** (4-F substituted) inhibited IDO1 levels as 100%, and 65%, respectivly, while compounds **19c** (3-Cl substituted), **19e** (3-F substituted) inhibited 49%, and 57% IDO1 expressions. This was consistent with structure-activity relationship (SAR) study reported by Qian and his colleagues in 2016[16]. These results confirmed the role of ion-dipole interaction between the substituent at *para*- position of the benzene ring in pocket B and Arg231. Noteworthy, **19d**, **19g** with 4-chloro, 4-bromo substituents presented the best potential activity with inhibitory percentages were 100%, and 99 %, respectivly, about equal to the positive control **IDO5L**. This may be because **19d**, **19g** had appropriate structures to interact with the active site of the enzyme IDO1.

5. CONCLUSION

Twenty-six novel 5/6-amino indazole derivatives having amide template were designed, synthesized, and confirmed the structures by IR, HR-MS, NMR spectra. An analysis of the *in vitro* IDO1 inhibition activity indicated that 13 synthesized compounds presented moderate to excellent activities with inhibitory percentages range from 49% to 100% at 1.0 mM. Especially, compound **19d** with chloro substituted group at the para- position on the benzene ring has the ability to inhibit IDO1 which is 100% as high as that of the positive control **IDO5L**. The study reconfirmed the role of a special halogen substituent at para- position for IDO1 inhibitory activity, and contributed new derivatives to the "IDO1 inhibitors bank" toward cancer treatment.

Conflict of interest
None to declare.

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NXH, TKN, V-HH, and P-TT conceived, planned, and carried out the synthetic experiments. TTLV. did the biological experiments. All authors contributied to the interpretation of the results, drafted manuscript, provided critical feedback, and helped shape the research, analysis results.

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