Design, synthesis and biological evaluation of novel 2- (indole arylamide) benzoic acid analogs as dual COX-2 / 5-LOX inhibitors

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Abstract

Recently, the drug discovery paradigm has evolved from single-target inhibition to a multi-target design concept. This study designed, synthesized, and evaluated a series of novel 2-(indole arylamide) benzoic acid analogs for their in vivo anti-inflammatory properties. Compounds **7f** and **7n** showed significant anti-inflammatory activity in a xylene-induced mouse model of auricular edema. Furthermore, **7f** and **7n** exhibited moderate COX-2 inhibitory activity (IC_{50} = 537 and 321.5 nM) than celecoxib (IC_{50} = 10.04 nM) in vitro, among which **7n** had higher COX-2 selectivity activity (selectivity index (COX-1/COX-2) = 7.89) and moderate 5-LOX inhibitory activity (IC_{50} = 222.1 nM). Compared to zileuton (IC_{50} = 36.46 nM), compound **7f** was identified as the most potent 5-LOX inhibitor (IC_{50} = 77.37 nM). According to the biological results, compounds **7f** and **7n** have better inhibitory activities on the production of NO and PGE_2 in LPS-induced RAW 264.7 cell macrophages than celecoxib and indomethacin. As demonstrated by docking studies, **7f** and **7n** have stronger interactions with key residues in the active pocket of COX-1 or COX-2, which is consistent with the activity results. Based on these results, further research into safer and more effective anti-inflammatory drugs might be possible using 2-(indole arylamide) benzoic acid analogs.

1. Introduction

Inflammation is a multifaceted pathophysiological process [1–3]. Physiological imbalances can result from an exaggerated inflammatory response[4]. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) exert antipyretic, analgesic, and anti-inflammatory effects by inhibiting cyclooxygenase (COX) enzyme-associated inflammatory cytokines synthesis[5]. In COX, there are two subtypes: COX-1 and COX-2. COX-1 is constitutively expressed in several tissues and has a key role in physiological homeostasis. COX-2 is an inducible enzyme whose expression is enhanced during tissue damage, inflammation, etc.[6–8]. However, there is a chance of severe gastrointestinal side effects due to persistent intake of non-selective COX inhibitors, such as indomethacin[9]. Application of selective COX-2 inhibitors such as celecoxib and rofecoxib prevents gastrointestinal side effects. It also decreases the biosynthesis of prostaglandin I_2 (PGI_2), which is responsible for promoting vasoconstriction and increased thromboxane A_2 (TXA) synthesis, thereby promoting platelet aggregation[10–12]. Other selective COX-2 inhibitors like rofecoxib and valdecoxib have been withdrawn from the market owing to ulcerative and cardiovascular side effects[13, 14]. In addition to the COX-mediated pathway, lipoxygenase (LOX) enzymes metabolize arachidonic acid (AA) to leukotrienes (LTs)[15, 16]. There is increased production of LTs due to COX inhibitors. When the activity of COXs is inhibited, AA is metabolized to LTs via 5-LOX pathway. LTs are thought to be crucial mediators of allergy and inflammatory diseases[18, 19]. Until now, the only approved treatment for asthma is zileuton, a 5-LOX inhibitor, but its side effects include hepatotoxicity and poor pharmacokinetics[17].

Selective COX-2 inhibitors cause cardiovascular risk, and the compensation mechanism of 5-LOX indicates that inhibiting any biosynthetic pathway might lead to the conversion of metabolism to other pathways. Therefore, COX-2 selective inhibitors with cardiovascular risk are not only related to PGI_2 and TXA_2 but also to the synthesis of LTs. Reduction in the synthesis of LTs and inhibition of the 5-LOX enzyme might reduce the potential cardiovascular risk of NSAIDs[20, 21]. Hence, dual COX-2/5-LOX inhibitors may prove to be effective as a strategy for developing new anti-inflammatory drugs with better safety by blocking the production of inflammatory mediators in the AA pathway.

The indole skeleton has various biological functions and is available as diverse natural products[22]. Various studies have proven NSAIDs with indole rings can reduce gastrointestinal side effects which is due to their potent COX-1 inhibitory activity[23–25]. A series of dual COX-2/5-LOX inhibitors having an indole ring with tosyl group at N-1 and dipeptide substituent at the C-3 position was designed and created by Singh et al. Among them, compound 1 exhibited potent COX-2 and 5-LOX inhibitory activity with IC_{50} values of 6.3 nM and 2.5 nM, respectively, which is superior to positive controls celecoxib and zileuton[26]. Reddy synthesized a series of compounds by modifying the structure of N-benzyl indole, in which compound 2 showed good COX and LOX dual inhibitory activity with IC50 values of COX-2 = 3.9 μM and 5-LOX = 94 μM, respectively[27]. Huang et al. obtained compound 3 by taking basic structures of 5-chloroindole-2-carboxylic acid and piperazine and introducing ligustrazine fragments with various biological functions. It has potent anti-inflammatory activity with IC_{50} values of 21.86 nM and 339 nM for COX-2/5L-LOX, respectively[28]. Besides, indazole also has a wide range of anti-inflammation activities, which has a similar structure to indole. For example, benzydamine is treated using acute or chronic inflammation and various inflammatory diseases (Fig. 1).

A class of compounds derived from N-Aryl anthranilic acid has analgesic, antipyretic and anti-inflammatory activities, and a variety of molecules with different biological functions have been developed from this class of compounds (Fig. 2)[29–31]. Han et al. designed the N-sulfonyl anthranilic acid compound **4** as an anti-inflammatory agent that directly binds to the active site of COX-2 and achieves 98% of anti-inflammatory effects on COX-2[32]. Narsinghani and Sharma reported the amide derivatives of meclofenamic acid (compound **5**) with an improved anti-inflammatory effect. The tested derivatives of meclofenamic acid selectively inhibited the inducible COX-2 isoenzyme when analyzed through the in vitro enzyme assays and in vivo studies on animal models[33]. Studies show that substituting various substituted aryl or heteroaryl moieties at the 2-position of anthranilic acid (2-aminobenzoic acid) significantly modulates its anti-inflammatory activity.[34] (Fig. 2).

We continued to work in this field with the new findings and designed the study. We synthesized a novel set of analogs derived from 2-(indole arylamide) benzoic acid as dual COX and LOX inhibitors with potent anti-inflammatory activity (Fig. 3). In order to obtain compounds with potent anti-inflammatory activity, we constructed a new pharmacophore model with three regions; the indole and/or indazole template, amide linker and hydrophobic region with formic acid. The most active compounds **7f** and **7n** were further tested for COX-1, COX-2, PGE_2, and NO inhibitory activities *in vitro* by evaluating their *in vivo* anti-

inflammatory activities. In addition, molecular model docking further studied the binding effect of the most active molecules on the active sites of COX-2.

2 Results And Discussion

2.1 Chemistry

The synthetic route of the target compound is depicted in **Scheme 1.** Methyl esterification was conducted as a key precursor due to the presence of two active sites in indole and indazole carboxylic acids. Then, the obtained methyl ester was reacted with benzyl chloride (C_7H_7Cl) in the presence of sodium hydride (NaH) in Dimethylformamide (DMF), in 65–86% yields, [28, 37, 38], respectively. Compound **4a-w** yielded 84–93% through a hydrolysis reaction. Furthermore, the acyl chloride compound **5a-w** was formed by treating the compound **4a-w** with oxalyl chloride and combining it with ethyl anthranilate in an alkaline environment to yield compound **6a-w** [39, 40]. Finally, the target compounds **7a-w** were hydrolyzed with 86–96% yield under weak base conditions. The techniques of NMR (^1H, ^{13}C) and HRMS were used to characterize the compounds.

Table 1
Biologically tested benzoic acid analog

Cpd	R_1	R_2	m.p (°C)	Yield (%)	Cpd	R_1	R_2	m.p (°C)	Yield (%)
7a	**H**	**F**	237.1–238.0	51	**7m**	**H**	**OCH_3**	204.5–205.9	40
7b	**H**	**Cl**	237.5–239.3	49	**7n**	**H**	**CH_3**	231.0–232.6	44
7c	**H**	**OCH_3**	206.1–207.8	43	**7o**	**H**	**CF_3**	231.0–232.6	46
7d	**H**	**CH_3**	222.3–223.8	50	**7p**	**Cl**	**F**	231.7–233.2	38
7e	**H**	**CF_3**	250.5–252.2	43	**7q**	**Cl**	**OCH_3**	227.8–229.5	40
7f	**Cl**	**F**	213.6–215.2	46	**7r**	**Cl**	**CH_3**	187.4–229.5	46
7g	**Cl**	**Cl**	234.2–235.4	44	**7s**	**Cl**	**CF_3**	245.2–247.0	36
7h	**Cl**	**OCH_3**	214.2–215.4	48	**7t**	**H**	**F**	223.3–224.8	50
7i	**Cl**	**CH_3**	227.2–228.9	88	**7u**	**H**	**OCH_3**	254.9–256.1	44
7j	**Cl**	**CF_3**	240.7–242.0	40	**7v**	**H**	**CH_3**	264.6–265.3	46
7k	**H**	**F**	228.4–230.1	39	**7w**	**H**	**CF_3**	224.5–226.0	41
7l	**H**	**Cl**	225.3–226.9	47					

2.2 Biological evaluation

2.2.1 *In vivo* Anti-inflammatory activity

The synthesized compounds were checked for their anti-inflammatory activity, and evaluation was done by taking indomethacin as positive control and utilizing the mice auricle edema model. Table 2 shows the experimental results expressed as means ± standard deviations (SDs). One-way analysis of variance (ANOVA) was performed on the control and the compound groups using SPSS 23.0 software. A significant difference was observed at $p < 0.05$.

Each test compound was injected by intragastric administration (the positive group was given indomethacin 10 mg/kg, and the compound group was given 5 mg/kg, respectively). Adaptive feeding was done for 6 days, and xylene was used to stimulate inflammation. The mice were sacrificed one hour later. Both the ears were checked for their weight, and calculations were done for the degree of swelling inhibition rate [28].

Compound **7a-w** was studied for *in vivo* anti-inflammatory activity, and the results showed that all compounds except **7p** had certain anti-inflammatory activity against swelling of ear swelling induced by xylene in mice. Compounds **7f** (40.35%) and **7n** (44.47%) had significant inhibitory activity compared with the control group ($p < 0.01$). A significant difference was not observed between **7n** and the positive control indomethacin.

Table 2
The percentages of edema inhibition of compounds**7a-w** band indomethacin

Compound	Dose levels (mg/kg)	left ear (mg)	Right ear (mg)	swelling degree (mg)	inhibition (%)
Blank	-	-	-	-	-
Control	-	9.6 ± 0.2	17.1 ± 1.2	7.5 ± 1.4	-
Indomethacin	10	9.5 ± 0.2	13.6 ± 1.4	4.0 ± 1.5**	46.47
7a	5	9.6 ± 0.2	15.3 ± 1.9	5.7 ± 2.0*▲	23.83
7b	5	9.6 ± 0.2	14.7 ± 1.3	5.1 ± 1.3**	32.22
7c	5	9.5 ± 0.2	15.7 ± 1.4	6.2 ± 1.4*▲▲	17.71
7d	5	9.6 ± 0.2	15.1 ± 1.3	5.5 ± 1.3**▲	26.90
7e	5	9.7 ± 0.3	14.8 ± 1.2	5.1 ± 1.1**	31.82
7f	5	9.5 ± 0.2	14.0 ± 1.3	4.5 ± 1.3**	40.35
7g	5	9.5 ± 0.1	14.8 ± 1.5	5.3 ± 1.5**	29.69
7h	5	9.5 ± 0.2	14.2 ± 1.0	4.6 ± 1.0**	38.22
7i	5	9.4 ± 0.2	15.1 ± 1.1	5.7 ± 1.0**▲▲	24.10
7j	5	9.5 ± 0.2	14.4 ± 1.2	4.8 ± 1.2**	35.55
7k	5	9.5 ± 0.1	16.1 ± 1.5	6.6 ± 1.5▲▲	12.12
7l	5	9.6 ± 0.2	14.4 ± 1.3	4.9 ± 1.2**	35.42
7m	5	9.5 ± 0.2	16.0 ± 1.3	6.5 ± 1.4▲▲	14.11
7n	5	9.7 ± 0.2	13.9 ± 1.0	4.2 ± 0.9**	44.47
7o	5	9.5 ± 0.1	16.3 ± 1.7	6.8 ± 1.7▲▲	9.32
7p	5	9.6 ± 0.1	17.4 ± 1.3	7.7 ± 1.3▲▲	-3.06
7q	5	9.7 ± 0.1	14.2 ± 1.2	4.5 ± 1.2**	39.55
7r	5	9.5 ± 0.1	14.9 ± 1.7	5.4 ± 1.7**	27.96
7s	5	9.5 ± 0.2	14.9 ± 1.3	5.4 ± 1.4**	28.63

*$p < 0.05$, **$p < 0.01$ vs model group;▲$p < 0.05$, ▲▲$p < 0.01$ vs indomethacin group

Compound	Dose levels (mg/kg)	left ear (mg)	Right ear (mg)	swelling degree (mg)	inhibition (%)
7t	5	9.7 ± 0.1	14.6 ± 1.3	5.0 ± 1.4**	33.56
7u	5	9.5 ± 0.1	14.2 ± 1.2	4.7 ± 1.3**	37.95
7v	5	9.6 ± 0.2	14.8 ± 1.3	5.2 ± 1.3**	30.49
7w	5	9.6 ± 0.1	15.8 ± 1.1	6.2 ± 1.1*▲▲	17.44
*p < 0.05, **p < 0.01 vs model group;▲p < 0.05, ▲▲p < 0.01 vs indomethacin group					

Swelling degree (mg) = V_R- V_L

Inhibition (%) = ((V_R- V_L) control - (V_R- V_L) treated)/(V_R- V_L) control * 100%

V_R= Average weight of the right ear; V_L= Average weight of the left ear

2.2.2 *In vitro* COX-1 and COX-2 inhibition assays

The synthesized compounds were tested for COX-1/COX-2 inhibitory activity by using COX-1 and COX-2 screening assay kits. Celecoxib, indomethacin, mefenamic acid, and zileuton were used as corresponding positive controls. The results were presented in the form of IC_{50} values for *in vitro* enzyme inhibition.

Compounds **7f** and **7n** both showed inhibitory activity at certain levels (IC_{50} = 537 and 321.5 nM, respectively) and selectivity (SI = 2.55 and 7.89, respectively) against COX-2 (Table 3). Out of both, compound **7n** showed the strongest COX-2 inhibitory activity and selectivity, and it showed better inhibitory activity than the positive controls indomethacin and mefenamic acid but less than the COX-2 selective inhibitor. The combination of indole skeleton and anthranilic acid from the inhibition results can increase the selectivity to COX-2 and reduce the inhibitory effect on COX-1, thereby helping mitigate serious gastrointestinal side effects.

2.2.3 *In vitro* 5-LOX inhibition assays

Similarly, 5-LOX inhibitory activity was evaluated on **7f** and **7n**, and Table 3 presents the obtained data. Zileuton and mefenamic acid were used as positive controls.

Table 3 confirms both **7f** and **7n** show potent 5-LOX inhibitory activity (IC_{50} = 77.37 and 222.1 nM, respectively). Compounds **7f** and **7n** are more potent than the positive control mefenamic acid (IC_{50} = 303.7 nM). Among these, compounds **7f** had stronger inhibitory activities (IC_{50} = 77.37 nM, respectively) than that of positive control zileuton (IC_{50} = 36.46 nM). Hence, these results indicate a potent and better inhibitory activity by the selected compounds against COX-2/5-LOX. The inhibition of COX-2/5-LOX may be one of the ways these compounds exert their biological functi

Table 3
IC_{50} values of compounds on inhibition of COX-1, COX-2 and 5-LOX activities

compounds	IC_{50} (nM)			
	COX-1	COX-2	SI [b]	5-LOX
7f	$1367 \pm 163.89^{**\#\#bb}$	$537 \pm 33.40^{**\#\#bb}$	2.55	77.37 ± 1.48^{aabb}
7n	$2537 \pm 202.06^{**\#\#bb}$	$321.5 \pm 38.89^{**\#\#bb}$	7.89	222.1 ± 21.00^{aabb}
Celecoxib	> 1000	10.04 ± 0.54	> 40	ND
Indomethacin	$293.7 \pm 16.79^{**}$	$14092 \pm 407.55^{**}$	0.02	ND
Zileuton	ND	ND	ND	36.46 ± 2.05
mefenamic acid	$43.41 \pm 2.7^{**}$	$3069 \pm 150.44^{**\#\#}$	0.01	303.7 ± 5.12^{aa}
a "**" vs Celecoxib $p < 0.01$, "##" vs Indomethacin $p < 0.01$, "aa"vs Zileuton $p < 0.01$, "bb"vs mefenamic acid $p < 0.01$.				

b SI: IC_{50} (COX-1)/IC_{50} (COX-2).

ND: not determined.

2.2.4 Assay for NO and PGE_2 inhibition

NO, and PGE_2 are the mediators of inflammatory response. We tested the ability of compounds (**7f** and **7n)** to inhibit the production of PGE_2 from arachidonic acid. RAW264.7 cells were treated with compounds **7f**, **7n**, celecoxib, indomethacin, and LPS having different doses for 24 h, and the cell culture medium was collected after 24 h to detect the expression of PGE_2 using a PGE_2 ELISA kit. Table 4 shows treatment of RAW264.7 cells with compounds **7f** (10 μM) or **7n** (10 μM) led to decreased production of PGE_2. They are better than indomethacin (10 μM) and celecoxib (10 μM) when compared with the control group. These results help conclude that compounds **7f** and **7n** may participate in the COX-2/PGE_2 pathway as a COX-2 inhibitor.

The essential roles of NO in inflammation and its association with COX-2 expression are well known. Hence, we used the Griess method to analyze the effects of compounds **7f** and **7n** to produce NO induced by LPS in RAW264.7 cells. Table 4 shows significant inhibition of NO and PEG_2 secretion at a concentration of 10 μM in RAW264.7 cells stimulated by LPS, and the inhibitory effect of compounds **7f** and **7n** on NO was stronger than that of the positive control compound indomethacin. Among these, compound **7f** was found to be better than celecoxib. These results confirmed a reduction in LPS-induced inflammatory response in RAW264.7 cells by compounds **7f** and **7n**.

Table 4
Potency of Inhibitors among the LPS induced RAW264.7 cells to secrete NO, PEG_2

compound	concentration	NO (μM)	PEG_2 (pg/mL)
celecoxib	10 μM	12.47 ± 1.60**	57.15 ± 4.16
Indomethacin	10 μM	17.05 ± 1.45	54.29 ± 4.93
7f	10 μM	11.54 ± 0.54**	47.04 ± 3.88
7n	10 μM	12.67 ± 0.83*	49.27 ± 1.92

2.2.5 *In vitro* cytotoxic activity

Further, a normal macrophage cell line (RAW264.7) was used to evaluate the cytotoxicity of compounds **7f** and **7n.** The results showed that no significant effect was seen on the survival of RAW264.7 cells when compounds **7f** and **7n** with doses below 10 μM (including 10 μM) were given (Fig. 4).

2.2.6 Molecular docking study

A molecular docking study was performed to investigate the possible binding conformations of compounds **7f** and **7n** with either COX-1 (PDB code 1PGF) or COX-2 (PDB code 1CX2) receptors, using AutoDock 4.2 modeling software. PYMOL tool was used to process the docking results and obtain the molecular chimeric maps, and analysis for the positions where the compounds formed hydrogen bonds and binding pockets were done [41–44].

Figure 5 (A) **7f** enters the COX-1 active pocket surrounded by TYR355, ARG120, SER530, and TYR385 [28, 45], and combines ARG120 with hydrogen bonds. In Fig. 5 (B), the combination product of compound **7f** and COX-2 enzyme was similar to that of COX-1. In contrast to COX-1's Ile523, the binding site for COX-2 has an extra side pocket due to the amino acid residue Val523, which enhances COX-2's active area by 20%. Moreover, COX-2 protein accommodates heavier structures and allows other interactions with amino acid residues such as Arg513 (substituted by a HIS513 in COX-1) [46]. According to the reports, the classic COX-2 inhibitors depend on ARG513 binding in the COX-2 extra pocket to provide their action and COX-1 selectivity. This binding is frequently accomplished by sulfone or sulfonamide groups. Figure 6 (A&C) shows a clear orientation of the benzyl and o-aminobenzoic acid moieties (**7f** and **7n**, respectively, within the additional side pockets of COX-2) with similarity to the assembly of the sulfonamide moiety of S-58 into the same side pocket. Further, compound **7f** forms three hydrogen bonds with HIS90, ARG513, and TYR355 residues, and VAL523 amino acid residue is present to enable it to enter the active side pocket and form hydrogen bonds with ARG513, which may lead the compound **7f** to have a certain activity to inhibit COX-2. Figure 6C shows compound **7n** forming three hydrogen bonds with top residues HIS90, ARG513, and IEU352 in the active pocket. When this compound **7n** is in the active site of COX-1 without side pockets (Fig. 5C), there was a loss in rotation and the above-mentioned interaction of hydrogen bonding too, which might be due to the strong inhibitory activity of **7n** against COX-2. Interestingly, it can

accommodate the benzyl and o-aminobenzoic acid of **7f** and **7n** with nearly identical binding throughout the COX-2 active site due to the bulky COX-2 active site. Hence, these interactions of the **7f** and **7n** in active sites may contribute to their remarkable inhibition activity against COX-2 and its selectivity.

3 Conclusions

The present study synthesized a series of novel 2-(indole arylamide) benzoic acid analogs as anti-inflammatory agents. Compounds **7f** and **7n** showed good anti-inflammatory properties in the mice auricle edema model and had been extensively studied for their mechanisms of action. They had no significant cytotoxicity on RAW264.7 at 10 μM, and were found to have an inhibitory effect on the COX-2/5-LOX enzyme. Among them, the inhibitory activities of **7f** and **7n** on COX-2 were 537 nM and 321.5 nM, respectively, while on 5-LOX, they were 77.31 nM and 222.1 nM. Therefore, it was confirmed that the compounds exert their anti-inflammatory activities by inhibiting the COX-2/5-LOX pathway. According to Guo Zongru's strategy of moderate inhibitions, due to the mutual restriction of COX-1 and COX-2 in the body, excessive inhibition of COX-2 will disrupt this kind of balance, resulting in unanticipated side effects [47]. Compounds **7f** and **7n** showed moderate inhibition of the COX-2/5-LOX and temperate selectivity for COX-2, making them potentially safer than celecoxib. In the docking study, by exploring the binding pattern of the compounds to the active site of the enzyme, it was found that the indole structure in the compounds **7f** and **7n** entered the COX-2 side pocket, which was similar to the sulfonamide group in S-58. Moreover, **7f** and **7n** were found to be superior to celecoxib and indomethacin at 10 μM in NO and PGE_2 inhibition assays. The results of this study, combined with the mice auricle edema and the enzyme immunosuppression experiments, led to the discovery of two promising compounds highly potent against COX and 5-LOX, paving the way for further discovery of safer and more effective anti-inflammatory drugs.

4. Experimental

4.1 Chemistry

Solvents and reagents for reaction were purchased from common commercial suppliers or purified using standard techniques. We tested all chemical reactions using TLC (thin layer board with 254 nm fluorescent indicator). Melting points are evaluated on the melting point apparatus (RDCSY-I). A Bruker 400 MHz spectrometer was used to record ^{1}H NMR spectra, and a 100 MHz spectrometer was used to record ^{13}C NMR spectra. Chemical shifts were represented in parts per million using tetramethylsilane as an internal reference and DMSO-d_6 as the solvent. HRMS spectra data were recorded with a Hewlett-Packard 1100 LC/MSD spectrometer.

4.2. General procedure for the synthesis of the compounds

4.2.1 Methyl 5-chloro-1*H*-indole-2-carboxylate (2b)

$SOCl_2$ (0.73 g, 6.13 mmol, 1.2 eq) was added to a solution of 5-Chloroindole-2-carboxylic acid (1 g, 5.11 mmol, 1 eq) in 15 mL of MeOH dropwise within 30 min at 0°C. Afterward, the reaction mixture was stirred at 75°C for 2 h. Subsequently, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography using ethylacetate/petroleum, giving intermediate **2b** as a yellow solid, yielding 91%. m.p. 211.9–213.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H, -N**H**-), 7.72 (d, *J* = 2.0 Hz, 1H, Ar-**H**), 7.47 (d, *J* = 8.8 Hz, 1H, Ar-**H**), 7.27 (dd, *J* = 8.8, 2.1 Hz, 1H, Ar-**H**), 7.17–7.10 (m, 1H, Ar-C**H=**), 3.88 (s, 3H, -O**CH$_3$**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 136.2, 129.0, 128.2, 125.3, 125.2, 121.6, 114.7, 107.7, 52.4; HRMS Calculated for **$C_{10}H_8ClNO_2$** $[M + H]^+$ 176.0712, found 176.0705.

Methyl 1 H **-indole-2-carboxylate (2a)**

Target compound **2a** were synthesized according to the synthetic procedure given above. white solid, yielding 93%. m.p. 149.3–151.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H, -N**H**-), 7.66 (d, *J* = 8.0 Hz, 1H, Ar-**H**), 7.47 (dd, *J* = 8.3, 0.7 Hz, 1H, Ar-**H**), 7.30–7.24 (m, 1H, Ar-**H**), 7.17 (d, *J* = 1.3 Hz, 1H, Ar-C**H=**), 7.11–7.05 (m, 1H, Ar-**H**), 3.88 (s, 3H, -O**CH$_3$**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 137.9, 127.5, 127.2, 125.1, 122.5, 120.7, 113.1, 108.2, 52.2; HRMS Calculated for **$C_{10}H_9NO_2$** $[M + H]^+$ 176.0712, found 176.0705.

Methyl 1 H **-indole-3-carboxylate (2c)**

Target compound **2c** were synthesized according to the synthetic procedure given above. white solid, yielding 90%. m.p. 147.6–149.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (s, 1H, =CH-N**H**-), 8.10 (s, 1H, =C**H**-NH-), 8.07–7.98 (m, 1H, Ar-**H**), 7.55–7.46 (m, 1H, Ar-**H**), 7.27–7.12 (m, 2H, Ar-**H**), 3.82 (s, 3H, -O**CH$_3$**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 136.9, 132.9, 126.1, 122.8, 121.7, 120.9, 112.8, 106.8, 51.1; HRMS Calculated for **$C_{10}H_9NO_2$** $[M + H]^+$ 176.0712, found 176.0706.

Methyl 5-chloro-1 H **-indole-3-carboxylate (2d)**

Target compound **2d** were synthesized according to the synthetic procedure given above. white solid, yielding 94%. m.p. 202.4–203.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H, =CH-N**H**-), 8.16 (d, *J* = 2.7 Hz, 1H, Ar-**H**), 7.97 (d, *J* = 2.0 Hz, 1H, =C**H**-NH-), 7.53–7.49 (m, 1H, Ar-**H**), 7.22 (dd, *J* = 8.6, 2.1 Hz, 1H, Ar-**H**), 3.82 (s, 3H, -O**CH$_3$**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 135.4, 134.4, 127.3, 1266, 122.9, 120.0, 114.5, 106.6, 51.3; HRMS Calculated for **$C_{10}H_8ClNO_2$** $[M + H]^+$ 210.0322, found 210.0316.

Methyl 1 H **-indazole-3-carboxylate (2e)**

Target compound **2e** were synthesized according to the synthetic procedure given above. white solid, yielding 96%. m.p. 164.9–166.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.94 (s, 1H, =N-N**H**-), 8.09 (d, *J* = 8.2 Hz, 1H, Ar-**H**), 7.67 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.45 (m, 1H, Ar-**H**), 7.34–7.29 (m, 1H, Ar-**H**), 3.93 (s, 3H, -O**CH$_3$**);

^{13}C NMR (100 MHz, DMSO-d_6) δ 163.2, 141.4, 135.5, 127.1, 123.3, 122.6, 121.4, 111.5, 52.1; HRMS Calculated for $\mathbf{C_9H_8N_2O_2}$ [M + H]$^+$ 177.0664, found 177.0657.

4.2.2 Methyl 5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxylate (3i)

NaH (0.13 g, 5.56 mmol, 1.2 eq) was added to a solution of 2b (0.97 g, 4.63 mmol, 1 eq) in 15 mL of DMF dropwise within 10 min at 0°C, and the mixture was stirred at room temperature for 30 min. Subsequently, 4-methylbenzyl chloride (0.78 g, 5.56 mmol, 1.2 eq) was dropwise added to the mixture, and the resulting mixture was stirred at room temperature for 30 min. This reaction was quenched with water. The resultant precipitate was filtered, washed with water, and dried under a vacuum to obtain a white solid, yielding 84%. m.p. 113.5–115.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 1.9 Hz, 1H, Ar-**H**), 7.61 (m,, 1H, Ar-**H**), 7.35–7.28 (m, 2H, Ar-**H**, Ar-C**H**=), 7.06 (m, 2H, Ar-**H**), 6.92 (d, J = 8.0 Hz, 2H, Ar-**H**), 5.80 (s, 2H, Ar-C$\mathbf{H_2}$), 3.84 (s, 3H, -O$\mathbf{CH_3}$), 2.21 (s, 3H, Ar-C$\mathbf{H_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 137.9, 136.8, 135.5, 129.5, 128.7, 127.0, 126.7, 125.9, 125.7, 122.0, 113.7, 110.5, 52.4, 47.5, 21.0; HRMS Calculated for $\mathbf{C_{18}H_{16}ClNO_2}$ [M + H]$^+$ 314.0948, found 314.0939.

Methyl 1-(4-methylbenzyl)-1 H **-indole-2-carboxylate (3d)**

Target compound **3d** were synthesized according to the synthetic procedure given above. white solid, yielding 75%. m.p. 61.2–62.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (d, J = 8.0 Hz, 1H, Ar-**H**), 7.38 (d, J = 0.6 Hz, 1H, Ar-**H**), 7.31 (d, J = 1.2 Hz, 1H, Ar-**H**), 7.18–7.11 (m, 1H, Ar-C**H**=), 7.06 (d, J = 7.9 Hz, 2H, Ar-**H**), 6.94 (d, J = 8.0 Hz, 2H, Ar-**H**), 5.81 (s, 2H, Ar-C$\mathbf{H_2}$), 3.83 (s, 3H, -O$\mathbf{CH_3}$), 2.21 (s, 3H, Ar-C$\mathbf{H_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 139.52, 136.7, 135.9, 129.5, 127.3, 126.8, 126.0, 125.7, 123.0, 121.3, 111.9, 111.1, 52.22, 47.3, 21.0; HRMS Calculated for $\mathbf{C_{18}H_{17}NO_2}$ [M + H]$^+$ 280.1338, found 280.1328.

Methyl 1-(4-fluorobenzyl)-1 H **-indole-3-carboxylate (3k)**

Target compound **3k** were synthesized according to the synthetic procedure given above. white solid, yielding 70%. m.p. 76.2–77.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H, =C**H**-NH-), 8.07–8.00 (m, 1H, Ar-**H**), 7.61–7.54 (m, 1H, Ar-**H**), 7.40–7.32 (m, 2H, Ar-**H**), 7.25–7.21 (m, 2H, Ar-**H**), 7.19–7.13 (m, 2H, Ar-**H**), 5.50 (s, 2H, Ar-C$\mathbf{H_2}$), 3.82 (s, 3H, -O$\mathbf{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.1(J_{C-F} = 242.4 Hz), 136.6, 136.0, 133.8(J_{C-F} = 3.1 Hz), 130.0(J_{C-F} = 8.3 Hz), 126.8, 123.1, 122.2, 121.2, 116.0(J_{C-F} = 21.3 Hz), 111.7, 106.4, 51.2, 49.3; HRMS Calculated for $\mathbf{C_{18}H_{17}NO_2}$ [M + H]$^+$ 284.1087, found 284.1078.

Methyl 5-chloro-1-(4-methylbenzyl)-1 H **-indole-3-carboxylate (3r)**

Target compound **3r** were synthesized according to the synthetic procedure given above. white solid, yielding 80%. m.p. 111.1–112.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (s, 1H, =C**H**-NH-), 7.98 (d, J = 1.9 Hz, 1H, Ar-**H**), 7.59 (d, J = 8.8 Hz, 1H, Ar-**H**), 7.24 (dd, J = 8.8, 2.1 Hz, 1H, Ar-**H**), 7.21–7.10 (m, 4H, Ar-**H**), 5.46

(s, 2H, Ar-C**H**$_2$), 3.83 (s, 3H, -O**CH**$_3$), 2.24 (s, 3H, Ar-C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 137.6, 137.3, 135.2, 134.2, 129.7, 127.8, 127.1, 123.1, 120.3, 113.6, 106.0, 51.4, 50.1, 21.1; HRMS Calculated for **$C_{18}H_{16}ClNO_2$** $[M + H]^+$ 314.0948, found 314.0937.

Methyl 1-(4-fluorobenzyl)-1 H **-indazole-3-carboxylate (3t)**

Target compound **3t** were synthesized according to the synthetic procedure given above. white solid, yielding 78%. m.p. 81.1–82.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, J = 8.2 Hz, 1H, Ar-**H**), 7.89 (d, J = 8.5 Hz, 1H, Ar-**H**), 7.50 (m, 1H, Ar-**H**), 7.39–7.31 (m, 3H, Ar-**H**), 7.20–7.12 (m, 2H. Ar-**H**), 5.79 (s, 2H, Ar-C**H**$_2$), 3.93 (s, 3H, -O**CH**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ, 162.8, 162.2(J_{C-F} = 242.4 Hz), 140.8, 134.8, 133.2(J_{C-F} = 3.0 Hz), 130.2(J_{C-F} = 8.3 Hz), 127.5, 123.8, 123.5, 121.8, 116.0(J_{C-F} = 21.4 Hz), 111.4, 52.3, 52.2; HRMS Calculated for **$C_{16}H_{13}FN_2O_2$** $[M + H]^+$ 285.1039, found 285.1033.

4.2.3 5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxylic acid (4i)

The above compound **3i** was dissolved in MeOH (20 mL) with the addition of 10% NaOH solution (15 mL) to the reaction mixture. The mixture was refluxed at 75°C for 1 h. After MeOH was removed under reduced pressure, the residue was taken into the water and acidified with concentrated hydrochloric acid. The resultant precipitate was filtered, washed with water, and dried under vacuum to obtain an intermediate **4i** as a white solid, yielding 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H, Ar-COO**H**), 7.78 (d, J = 2.0 Hz, 1H, Ar-**H**), 7.58 (d, J = 9.0 Hz, 1H, Ar-**H**), 7.30–7.28 (m, 2H, Ar-C**H**=, Ar-**H**), 7.07 (d, J = 7.9 Hz, 2H, Ar-**H**), 6.92 (d, J = 8.0 Hz, 2H, Ar-**H**), 5.83 (s, 2H, Ar-C**H**$_2$), 2.22 (s, 3H, Ar-C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.1, 137.7, 136.7, 135.7, 130.1, 129.5, 127.1, 126.7, 125.6, 125.3, 121.8, 113.6, 110.2, 47.3, 21.1; HRMS Calculated for $C_{17}H_{14}ClNO_2$ $[M-H]^-$ 298.0635, found 298.0629.

1-(4-methylbenzyl)-1 H **-indole-2-carboxylic acid (4d)**

Target compound **4d** were synthesized according to the synthetic procedure given above. white solid, yielding 91%. m.p. 171.0–172.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H, -COO**H**), 7.70 (d, J = 8.0 Hz, 1H, Ar-**H**), 7.52 (d, J = 8.4 Hz, 1H, Ar-**H**), 7.33 (s, 1H, Ar-C**H**=), 7.31–7.25 (m, 1H, Ar-**H**), 7.12 (t, J = 7.3 Hz, 1H, Ar-**H**), 7.06 (d, J = 7.9 Hz, 2H, Ar-**H**), 6.95 (d, J = 8.0 Hz, 2H, Ar-**H**), 5.84 (s, 2H, Ar-C**H**$_2$), 2.21 (s, 3H, Ar-C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4, 139.4, 136.6, 136.1, 129.4, 128.6, 126.8, 126.1, 125.3, 122.8, 121.1, 111.8, 110.8, 47.1, 26.8, 21.1; HRMS Calculated for **$C_{17}H_{15}NO_2$** $[M-H]^-$ 264.1025, found 264.1027.

1-(4-fluorobenzyl)-1 H **-indole-3-carboxylic acid (4k)**

Target compound **4k** were synthesized according to the synthetic procedure given above. white solid, yielding 90%. m.p. 197.2–198.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H, -COO**H**), 8.25 (s, 1H, N-C**H**=), 8.08–8.01 (m, 1H, Ar-**H**), 7.55 (m, 1H, Ar-**H**), 7.39–7.32 (m, 2H, Ar-**H**), 7.23–7.12 (m, 4H, Ar-**H**), 5.49

(s, 2H, Ar-C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d_6)) δ 166.1, 162.1(J_{C-F} = 242.4 Hz), 136.7, 135.9, 133.9(J_{C-F} = 3.0 Hz), 130.0(J_{C-F} = 8.3 Hz), 127.1, 122.9, 121.9, 121.4, 115.9(J_{C-F} = 21.4 Hz), 111.5, 107.5, 49.2; HRMS Calculated for **$C_{16}H_{12}FNO_2$** [M-H]$^-$ 268.0774, found 268.0773.

5-chloro-1-(4-methylbenzyl)-1 H **-indole-3-carboxylic acid (4r)**

Target compound **4r** were synthesized according to the synthetic procedure given above. white solid, yielding 95%. m.p. 210.5–211.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H, -COO**H**), 8.28 (s, 1H, N-C**H**=), 8.00 (d, *J* = 2.0 Hz, 1H, Ar-**H**), 7.56 (d, *J* = 8.8 Hz, 1H, Ar-**H**), 7.22 (dd, *J* = 8.8, 2.1 Hz, 1H, Ar-**H**), 7.20–7.10 (m, 4H, Ar-**H**), 5.44 (s, 2H, Ar-C**H**$_2$), 2.24 (s, 3H, Ar-C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.7, 137.5, 137.2, 135.3, 134.3, 129.7, 128.3, 127.8, 126.8, 122.8, 120.4, 113.4, 107.0, 50.0, 21.1; HRMS Calculated for **$C_{17}H_{14}ClNO_2$** [M-H]$^-$ 298.0635, found 298.0638.

1-(4-fluorobenzyl)-1H-indazole-3-carboxylic acid (4t)

Target compound **4t** were synthesized according to the synthetic procedure given above. white solid, yielding 92%. m.p. 193.5–194.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.11 (s, 1H, -COO**H**), 8.11 (d, *J* = 8.2 Hz, 1H, Ar-**H**), 7.86 (d, *J* = 8.5 Hz, 1H, Ar-**H**), 7.51–7.44 (m, 1H, Ar-**H**), 7.38–7.29 (m, 3H, Ar-**H**), 7.16 (m, 2H, Ar-**H**), 5.77 (s, 2H, Ar-C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 162.1(J_{C-F} = 242.4 Hz), 140.9, 135.8, 133.4(J_{C-F} = 3.0 Hz), 130.2(J_{C-F} = 8.2 Hz), 127.3, 123.7, 123.5, 122.1, 116.0(J_{C-F} = 21.4 Hz), 111.2, 52.2; HRMS Calculated for **$C_{15}H_{11}FN_2O_2$** [M-H]$^-$ 269.0727, found 269.0733.

4.2.4 Ethyl 2-(5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxamido)benzoate (6i)

Under ice bath conditions, oxalyl chloride (0.85 g, 6.68 mmol, 2 eq) was slowly added dropwise to a solution of **4i** (1 g, 3.34 mmol, 1 eq) and DMF (3d) in DCM (20 mL). Once the dropwise addition was complete, the reaction was carried out for 10 min, and then continued at room temperature for 30 min. The resultant mixture was concentrated under reduced pressure to obtain 5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carbonyl chloride as a yellow solid, which was used for the further reaction without purification. A solution of ethyl anthranilate (0.55 g, 3.34 mmol, 1 eq) and pyridine (0.79 g, 10.02 mmol, 3 eq) at 0°C in DCM (10 mL) was added to the prepared 5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carbonyl chloride, which was stirred for 10 min and then at room temperature for 1 h. The reaction was monitored by TLC. A saturated solution of citric acid and brine (3 × 20 mL) was used to wash the organic phase, followed by drying over Na_2SO_4, filtration, and concentration under reduced pressure. The residue was purified by flash column chromatography on silica gel (petroleum/ethylacetate) to obtain the intermediate compound **6i** as a white solid, yielding 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H, CON**H**), 8.49–8.40 (m, 1H, Ar-**H**), 8.02 (dd, *J* = 8.0, 1.5 Hz, 1H, Ar-**H**), 7.88 (d, *J* = 2.0 Hz, 1H, Ar-**H**), 7.71–7.64 (m, 1H, Ar-**H**), 7.62 (d, *J* = 8.9 Hz, 1H, Ar-**H**), 7.36–7.21 (m, 3H, Ar-**H**, Ar-C**H**=), 7.03 (m, 4H, Ar-**H**), 5.86 (s, 2H, Ar-C**H**$_2$), 4.36 (q, *J* = 7.1 Hz, 2H, CH_3C**H**$_2$-), 2.21 (s, 3H, Ar-C**H**$_3$), 1.33 (t, *J* = 7.1 Hz, 3H, C**H**$_3CH_2$-); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 168.0, 160.2, 140.2, 137.6, 136.8, 135.7, 134.7, 133.1, 131.2, 129.5, 127.2, 126.9, 125.8, 125.2, 124.0, 121.7, 121.4, 118.0, 113.5, 106.0, 61.9, 47.5, 21.1, 14.5; HRMS Calculated for **$C_{26}H_{23}ClN_2O_3$** $[M + H]^+$ 447.1475, found 447.1465.

Ethyl 2-(1-(4-methylbenzyl)-1 H **-indole-2-carboxamido)benzoate (6d)**

Target compound **6d** were synthesized according to the synthetic procedure given above. white solid, yielding 75%. m.p. 137.4–139.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H, CONH), 8.51 (d, J = 8.4 Hz, 1H, Ar-H), 8.03 (dd, J = 7.9, 1.2 Hz, 1H, Ar-H), 7.78 (d, J = 7.9 Hz, 1H, Ar-H), 7.70–7.63 (m, 1H, Ar-H), 7.57 (d, J = 8.4 Hz, 1H, Ar-H), 7.35 (s, 1H, Ar-CH=), 7.33–7.21 (m, 2H, Ar-H), 7.16 (t, J = 7.5 Hz, 1H, Ar-H), 7.03 (m, 4H, Ar-H), 5.87 (s, 2H, Ar-CH_2), 4.37 (q, J = 7.1 Hz, 2H, CH_3CH_2-), 2.21 (s, 3H, Ar-CH_3), 1.33 (t, J = 7.1 Hz, 3H, CH_3CH_2-); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.1, 160.5, 140.6, 139.2, 136.6, 136.0, 134.8, 131.7, 131.2, 129.5, 127.0, 126.2, 125.2, 123.8, 122.7, 121.3, 121.1, 117.5, 111.8, 106.5, 61.9, 47.3, 21.1, 14.45; HRMS Calculated for **$C_{26}H_{24}N_2O_3$** $[M + H]^+$ 413.1865, found 413.1858.

Ethyl 2-(1-(4-fluorobenzyl)-1 H **-indole-3-carboxamido)benzoate (6k)**

Target compound **6d** were synthesized according to the synthetic procedure given above. white solid, yielding 70%. m.p. 137.4–139.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H, CONH), 8.59 (d, J = 8.4 Hz, 1H, Ar-H), 8.26 (s, 1H, N-CH=), 8.25–8.20 (m, 1H, Ar-H), 8.01 (dd, J = 7.9, 1.2 Hz, 1H, Ar-H), 7.68–7.62 (m, 1H, Ar-H), 7.62–7.55 (m, 1H, Ar-H), 7.39–7.32 (m, 2H, Ar-H), 7.28–7.22 (m, 2H, Ar-H), 7.21–7.15 (m, 3H, Ar-H), 5.57 (s, 2H, Ar-CH_2), 4.35 (q, J = 7.1 Hz, 2H, CH_3CH_2-), 1.31 (t, J = 7.1 Hz, 3H, CH_3CH_2-); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.1, 163.0, 162.1(J_{C-F} = 242.4 Hz), 141.5, 136.8, 134.6, 133.8(J_{C-F} = 3.0 Hz), 132.4, 131.1, 129.9(J_{C-F} = 8.2 Hz), 126.6, 123.2, 122.8, 122.0, 121.4, 121.2, 116.8, 116.0(J_{C-F} = 21.4 Hz), 111.7, 111.1, 61.7, 49.3, 14.5; HRMS Calculated for **$C_{25}H_{21}FN_2O_3$** $[M + H]^+$ 417.1614, found 417.1606.

Ethyl 2-(5-chloro-1-(4-methylbenzyl)-1 H **-indole-3-carboxamido)benzoate (6r)**

Target compound **6r** were synthesized according to the synthetic procedure given above. white solid, yielding 77%. m.p. 123.7–125.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 1H, CONH), 8.50 (d, J = 8.1 Hz, 1H, Ar-H), 8.31 (s, 1H, N-CH=), 8.21 (d, J = 1.5 Hz, 1H, Ar-H), 8.00 (d, J = 6.8 Hz, 1H, Ar-H), 7.71–7.58 (m, 2H, Ar-H), 7.31–7.11 (m, 6H, Ar-H), 5.54 (s, 2H, Ar-CH_2), 4.35 (m, 2H, CH_3CH_2-), 2.26 (s, 3H, Ar-CH_3), 1.31 (t, J = 7.0 Hz, 3H, CH_3CH_2-); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.1, 162.6, 141.1, 137.5, 135.4, 134.5, 134.3, 133.6, 131.1, 129.8, 127.9, 127.7, 126.8, 123.2, 123.1, 121.5, 120.6, 117.4, 113.4, 110.4, 61.7, 50.1, 21.1, 14.5; HRMS Calculated for **$C_{26}H_{23}ClN_2O_3$** $[M + H]^+$ 447.1475, found 447.1466.

Ethyl 2-(1-(4-fluorobenzyl)-1 H **-indazole-3-carboxamido)benzoate (6t)**

Target compound **6t** were synthesized according to the synthetic procedure given above. white solid, yielding 73%. m.p. 123.7–125.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H, CONH), 8.82 (d, J = 7.8

Hz, 1H, Ar-**H**), 8.27 (d, *J* = 8.1 Hz, 1H, Ar-**H**), 8.05 (dd, *J* = 8.0, 1.5 Hz, 1H, Ar-**H**), 7.90 (d, *J* = 8.6 Hz, 1H, Ar-**H**), 7.71–7.65 (m, 1H, Ar-**H**), 7.54–7.42 (m, 3H, Ar-**H**), 7.36 (t, *J* = 7.4 Hz, 1H, Ar-**H**), 7.20 (m, 3H, Ar-**H**), 5.81 (s, 2H, Ar-C**H**$_2$), 4.41 (q, *J* = 7.1 Hz, 2H, $CH_3C\mathbf{H}_2$-), 1.36 (t, *J* = 7.1 Hz, 3H, $\mathbf{H}_3CH_2$-); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.6, 162.2(J_{C-F} = 242.4 Hz), 160.8, 141.3, 140.8, 137.7, 134.9, 133.1(J_{C-F} = 3.1 Hz), 131.4, 130.3(J_{C-F} = 8.4 Hz), 127.7, 123.7, 123.3, 122.9, 122.1, 120.6, 116.4, 116.0(J_{C-F} = 21.4 Hz), 111.3, 61.9, 52.2, 14.5; HRMS Calculated for **$C_{24}H_{20}FN_3O_3$** [M + H]$^+$ 418.1567, found 418.1553.

4.2.5 2-(5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxamido)benzoic acid (7i)

The above compound **6i** was dissolved in MeOH:THF solution (2:1) (20 mL), and 10% LiOH solution (15 mL) was added to the reaction mixture. The mixture was refluxed at 50°C for 2 h. After removing the solution under reduced pressure, the residue was placed in water and acidified with concentrated hydrochloric acid. The obtained precipitate was filtered, washed with water, and dried under a vacuum to obtain the target compound **7i** as an orange solid, yielding 88%; m.p, 227.2–228.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.84 (s, 1H, Ar-COO**H**), 12.30 (s, 1H, CON**H**), 8.65 (d, *J* = 8.0 Hz, 1H, Ar-**H**), 8.07 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.76 (d, *J* = 7.9 Hz, 1H, Ar-**H**), 7.69–7.61 (m, 1H, Ar-**H**), 7.58 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.31 (s, 1H, Ar-C**H**=), 7.24–7.13 (m, 2H, Ar-**H**), 7.07-7.00 (m 4H, Ar-**H**), 5.88 (s, 2H, Ar-C**H**$_2$), 2.21 (s, 3H, Ar-C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 160.5, 141.5, 139.2, 136.6, 136.0, 134.8, 131.9, 131.8, 129.5, 127.0, 126.2, 125.1, 123.3, 122.6, 121.3, 120.1, 116.8, 111.8, 106.4, 47.3, 21.1; HRMS Calculated for **$C_{24}H_{19}ClN_2O_3$** [M-H]$^-$ 417.1006, found 417.0993.

2-(1-(4-fluorobenzyl)-1 H **-indole-2-carboxamido)benzoic acid (7a)**

Target compound **7a** were synthesized according to the synthetic procedure given above. Light yellow solid; yielding 51%; m.p, 237.1–238.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.82 (s, 1H, Ar-COO**H**), 12.26 (s, 1H, CON**H**), 8.64 (d, *J* = 7.8 Hz, 1H, Ar-**H**), 8.07 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.77 (d, *J* = 7.9 Hz, 1H, Ar-**H**), 7.69–7.63 (m, 1H, Ar-**H**), 7.60 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.34–7.30 (m, 2H, Ar-**H**, Ar-C**H**=), 7.25–7.14 (m, 4H, Ar-**H**), 7.14–7.07 (m, 2H, Ar-**H**), 5.91 (s, 2H, Ar-C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 161.7 (J_{C-F} = 242 Hz), 160.4, 141.4, 139.2, 135.2 (J_{C-F} = 3 Hz), 134.8, 131.8, 131.8, 129.1 (J_{C-F} = 8 Hz), 126.2, 125.3, 123.4, 122.7, 121.4, 120.2, 116.8, 115.7 (J_{C-F} = 21 Hz), 111.7, 106.5, 46.9; HRMS Calculated for **$C_{23}H_{17}FN_2O_3$** [M-H]$^-$ 387.1145, found 387.1147.

2-(1-(4-chlorobenzyl)-1 H **-indole-2-carboxamido)benzoic acid (7b)**

Target compound **7b** were synthesized according to the synthetic procedure given above. white solid, yielding 49%. m.p. 237.5–239.3 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.82 (s, 1H, Ar-COO**H**), 12.27 (s, 1H, CON**H**), 8.62 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 8.06 (dd, *J* = 8.0, 1.2 Hz, 1H, Ar-**H**), 7.77 (d, *J* = 7.9 Hz, 1H, Ar-**H**), 7.64 (t, *J* = 7.9 Hz, 1H, Ar-**H**), 7.57 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.35–7.28 (m, 4H, Ar-**H**, Ar-C**H**=), 7.23–7.13 (m, 4H, Ar-**H**), 5.90 (s, 2H, Ar-C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 160.4, 141.4, 139.2, 138.1, 134.8, 132.1,

131.8, 131.7, 128.9, 128.8, 126.2, 125.3, 123.4, 122.7, 121.5, 120.2, 116.8, 111.6, 106.5, 47.0; HRMS Calculated for $C_{23}H_{17}ClN_2O_3$ $[M-H]^-$ 403.0849, found 403.0851.

2-(1-(4-methoxybenzyl)-1 H -indole-2-carboxamido)benzoic acid (7c)

Target compound **7c** were synthesized according to the synthetic procedure given above. white solid, yielding 43%. m.p. 206.1–207.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.83 (s, 1H, Ar-COO**H**), 12.26 (s, 1H, CON**H**), 8.67 (d, *J* = 7.8 Hz, 1H, Ar-**H**), 8.08 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.76 (d, *J* = 7.9 Hz, 1H, Ar-**H**), 7.69–7.64 (m, 1H, Ar-**H**), 7.62 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.36–7.27 (m, 2H, Ar-**H**, Ar-C**H**=), 7.24–7.08 (m, 4H, Ar-**H**), 6.82 (d, *J* = 8.7 Hz, 2H, Ar-**H**), 5.85 (s, 2H, Ar-CH_2), 3.67 (s, 3H, Ar-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 160.5, 158.8, 141.5, 139.2, 134.9, 131.8, 130.9, 128.5, 126.2, 125.1, 123.4, 122.6, 121.3, 120.2, 116.7, 114.3, 111.8, 106.4, 55.4, 46.9; HRMS Calculated for $C_{24}H_{20}N_2O_4$ $[M-H]^-$ 399.1345, found 399.1345.

2-(1-(4-methylbenzyl)-1 H -indole-2-carboxamido)benzoic acid (7d)

Target compound **7d** were synthesized according to the synthetic procedure given above. Light yellow solid, yielding 50%. m.p. 222.3–223.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H,CON**H**), 8.65 (d, *J* = 8.3 Hz, 1H, Ar-**H**), 8.08 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.76 (d, *J* = 7.9 Hz, 1H, Ar-**H**), 7.67–7.60 (m, 1H, Ar-**H**), 7.57 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.31 (s, 1H, Ar-C**H**=), 7.31–7.28 (m 1H, Ar-**H**) 7.23–7.12 (m, 2H, Ar-**H**), 7.07-7.00 (m, 4H, Ar-**H**), 5.88 (s, 2H, Ar-CH_2), 2.21 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 160.5, 141.5, 139.2, 136.6, 136.0, 134.7, 131.9, 131.8, 129.5, 127.0, 126.2, 125.1, 123.3, 122.6, 121.3, 120.1, 117.0, 111.8, 106.4, 47.3, 21.1; HRMS Calculated for $C_{24}H_{20}N_2O_3$ $[M-H]^-$ 383.1396, found 383.1399.

2-(1-(4-(trifluoromethyl)benzyl)-1 H -indole-2-carboxamido)benzoic acid (7e)

Target compound **7e** were synthesized according to the synthetic procedure given above. white solid, yielding 43%. m.p. 250.5–252.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.85 (s, 1H, Ar-COO**H**), 12.30 (s, 1H,CON**H**), 8.60 (d, *J* = 7.8 Hz, 1H, Ar-**H**), 8.07 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.80 (d, *J* = 7.9 Hz, 1H, Ar-**H**), 7.70–7.60 (m, 3H, Ar-**H**), 7.57 (d, *J* = 8.4 Hz, 1H, Ar-**H**), 7.37 (s, 1H, Ar-C**H**=), 7.35–7.26 (m, 3H, Ar-**H**), 7.22–7.18 (m, 2H, Ar-**H**), 6.02 (s, 2H, Ar-CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 160.3, 144.0, 141.4, 139.2, 134.8, 131.8, 131.8, 128.1(J_{C-F} = 31.6 Hz), 127.5, 126.2, 125.9(J_{C-F} = 3.8 Hz), 125.5, 124.7(J_{C-F} = 270.3 Hz), 123.4, 122.8, 121.6, 120.2, 116.8, 111.5, 106.6, 47.4; HRMS Calculated for $C_{24}H_{17}F_3N_2O_3$ $[M-H]^-$ 437.1113, found 437.1118.

2-(5-chloro-1-(4-fluorobenzyl)-1 H -indole-2-carboxamido)benzoic acid (7f)

Target compound **7f** were synthesized according to the synthetic procedure given above. orange solid, yielding 46%. m.p. 213.6–215.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H, CON**H**), 8.60 (d, *J* = 8.0 Hz,1H, Ar-**H**), 8.06 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.86 (d, *J* = 2.0 Hz, 1H, Ar-**H**), 7.67–7.61 (m, 2H, Ar-**H**),

7.34–7.27 (m, 2H, Ar-**H**, Ar-C**H**=), 7.24–7.14 (m, 3H, Ar-**H**), 7.13–7.06 (m, 2H, Ar-**H**), 5.89 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 161.8(J_{C-F} = 241.6 Hz), 160.0, 141.2, 137.5, 134.9(J_{C-F} = 3.0 Hz), 134.8, 133.1, 131.8, 129.0(J_{C-F} = 8.2 Hz), 127.3, 126.0, 125.3, 123.6, 121.7, 120.3, 117.0, 115.8(J_{C-F} = 21.3 Hz), 113.5, 105.9, 47.1. HRMS Calculated for $\mathbf{C_{23}H_{16}ClFN_2O_3}$ [M-H]$^-$ 421.0755, found 421.0759.

2-(5-chloro-1-(4-chlorobenzyl)-1H-indole-2-carboxamido)benzoic acid (7g)

Target compound **7g** were synthesized according to the synthetic procedure given above. orange solid, yielding 44%. m.p. 234.2–235.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H, CON**H**), 8.60 (d, J = 8.4 Hz, 1H, Ar-**H**), 8.07 (dd, J = 7.9, 1.3 Hz, 1H, Ar-**H**), 7.88 (d, J = 1.9 Hz, 1H, Ar-**H**), 7.67–7.60 (m, 2H, Ar-**H**), 7.35–7.30 (m, 4H, Ar-**H**, Ar-C**H**=), 7.21 (t, J = 7.6 Hz, 1H, Ar-**H**), 7.12 (d, J = 8.4 Hz, 2H, Ar-**H**), 5.91 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 156.0, 141.2, 137.8, 137.6, 134.7, 133.1, 132.2, 131.8, 129.0, 128.8, 127.3, 126.0, 125.3, 123.5, 121.8, 120.3, 117.2, 113.4, 105.9, 47.2; HRMS Calculated for $\mathbf{C_{23}H_{16}Cl_2N_2O_3}$ [M-H]$^-$ 437.0460, found 437.0458.

2-(5-chloro-1-(4-methoxybenzyl)-1 H **-indole-2-carboxamido)benzoic acid (7h)**

Target compound **7h** were synthesized according to the synthetic procedure given above. white solid, yielding 48%. m.p. 214.2–215.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H, CON**H**), 8.63 (d, J = 8.4 Hz, 1H, Ar-**H**), 8.06 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.84 (d, J = 1.9 Hz, 1H, Ar-**H**), 7.68–7.59 (m, 2H, Ar-**H**), 7.30 (dd, J = 8.9, 2.1 Hz, 1H, Ar-**H**), 7.26 (s, 1H, Ar-C**H**=), 7.24–7.18 (m, 1H, Ar-**H**), 7.08 (d, J = 8.7 Hz, 2H, Ar-**H**), 6.85–6.79 (m, 2H, Ar-**H**), 5.83 (s, 2H, Ar-C$\mathbf{H_2}$), 3.66 (s, 3H, Ar-O$\mathbf{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 160.2, 158.9, 141.2, 137.5, 134.8, 133.3, 131.8, 130.6, 128.4, 128.2, 127.3, 125.8, 125.1, 123.6, 121.7, 120.3, 117.0, 114.4, 113.6, 55.5, 47.2; HRMS Calculated for $\mathbf{C_{24}H_{19}ClN_2O_4}$ [M-H]$^-$ 433.0955, found 433.0941.

2-(5-chloro-1-(4-(trifluoromethyl)benzyl)-1 H **-indole-2-carboxamido)benzoic acid (7j)**

Target compound **7j** were synthesized according to the synthetic procedure given above. light yellow solid, yielding 40%. m.p. 240.7–242.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.87 (s, 1H, Ar-COO**H**), 12.32 (s, 1H, CON**H**), 8.57 (d, J = 8.4 Hz, 1H, Ar-**H**), 8.06 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.90 (d, J = 1.9 Hz, 1H, Ar-**H**), 7.69–7.57 (m, 4H, Ar-**H**), 7.34–7.31 (m, 2H, Ar-**H**, Ar-C**H**=), 7.26 (d, J = 8.1 Hz, 2H, Ar-**H**), 7.23–7.18 (m, 1H, Ar-**H**), 6.01 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 159.9, 143.6, 141.2, 137.6, 134.8, 133.1, 131.8, 128.2(J_{C-F} = 31.4 Hz), 127.5, 127.3, 126.1, 125.9(J_{C-F} = 3.8 Hz), 125.4, 124.7(J_{C-F} = 270.3 Hz), 123.6, 121.8, 120.3, 117.0, 113.3, 106.0, 47.6; HRMS Calculated for $\mathbf{C_{24}H_{16}ClF_3N_2O_3}$ [M-H]$^-$ 471.0723, found 471.0706.

2-(1-(4-fluorobenzyl)-1 H **-indole-3-carboxamido)benzoic acid (7k)**

Target compound **7k** were synthesized according to the synthetic procedure given above. white solid, yielding 39%. m.p. 228.4–230.1 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 1H, Ar-COO**H**), 11.83 (s, 1H, CON**H**), 8.75 (d, J = 7.9 Hz, 1H, Ar-**H**), 8.29–8.22 (m, 2H, Ar-**H**, N-C**H**=), 8.05 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.66–7.61 (m, 2H, Ar-**H**), 7.39–7.35 (m, 2H, Ar-**H**), 7.29–7.12 (m, 5H, Ar-**H**), 5.57 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 163.0, 162.1(J_{C-F} = 242.3 Hz), 142.4, 136.8, 134.7, 133.9(J_{C-F} = 2.9 Hz), 132.7, 131.7, 129.9(J_{C-F} = 8.3 Hz), 126.3, 123.2, 122.4, 122.0, 121.2, 120.4, 116.1, 116.0(J_{C-F} = 21.5 Hz), 111.7, 111.5, 49.3; HRMS Calculated for $\mathbf{C_{23}H_{17}FN_2O_3}$ [M-H]$^-$ 387.1145, found 387.1137.

2-(1-(4-chlorobenzyl)-1 H **-indole-3-carboxamido)benzoic acid (7l)**

Target compound **7l** were synthesized according to the synthetic procedure given above. white solid, yielding 47%. m.p. 225.3–226.9 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.71 (s, 1H, Ar-COO**H**), 11.82 (s, 1H, CON**H**), 8.75 (d, J = 7.8 Hz, 1H, Ar-**H**), 8.29–8.22 (m, 2H, Ar-**H**, N-C**H**=), 8.05 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.67–7.61 (m, 1H, Ar-**H**), 7.59–7.57 (m, 1H, Ar-**H**), 7.44–7.38 (m, 2H, Ar-**H**), 7.31 (d, J = 8.5 Hz, 2H, Ar-**H**), 7.28–7.20 (m, 2H, Ar-**H**), 7.18–7.13 (m, 1H, Ar-**H**), 5.58 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.6, 162.9, 142.4, 136.9, 136.8, 134.7, 132.8, 131.7, 129.6, 129.2, 126.3, 123.2, 122.5, 122.0, 121.2, 120.4, 116.0, 111.7, 111.5, 49.3; HRMS Calculated for $\mathbf{C_{23}H_{17}ClN_2O_3}$ [M-H]$^-$ 403.0849, found 403.0841.

2-(1-(4-methoxybenzyl)-1 H **-indole-3-carboxamido)benzoic acid (7m)**

Target compound **7m** were synthesized according to the synthetic procedure given above. white solid, yielding 40%. m.p. 204.5–205.9 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 1H, Ar-COO**H**), 11.81 (s, 1H, CON**H**), 8.75 (d, J = 7.8 Hz, 1H, Ar-**H**), 8.24–8.21 (m, 2H, Ar-**H**, N-C**H**=), 8.05 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.66–7.62 (m, 2H, Ar-**H**), 7.30–7.20 (m, 4H, Ar-**H**), 7.15 (t, J = 7.4, 1H, Ar-**H**), 6.90 (d, J = 8.7 Hz, 2H, Ar-**H**), 5.48 (s, 2H, Ar-C$\mathbf{H_2}$), 3.71 (s, 3H, Ar-OC$\mathbf{H_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.6, 163.0, 159.3, 142.5, 136.9, 134.7, 132.6, 131.7, 129.5, 129.3, 126.3, 123.1, 122.4, 121.9, 121.1, 120.4, 116.0, 114.6, 111.8, 111.2, 55.5, 49.6; HRMS Calculated for $\mathbf{C_{24}H_{20}NO_4}$ [M-H]$^-$ 399.1345, found 399.1340.

2-(1-(4-methylbenzyl)-1 H **-indole-3-carboxamido)benzoic acid (7n)**

Target compound **7n** were synthesized according to the synthetic procedure given above. white solid, yielding 44%. m.p. 231.0–232.6 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.71 (s, 1H, Ar-COO**H**), 11.81 (s, 1H, CON**H**), 8.75 (dd, J = 8.4, 0.7 Hz, 1H, Ar-**H**), 8.27–8.19 (m, 2H, Ar-**H**, N-C**H**=), 8.05 (dd, J = 8.0, 1.6 Hz, 1H, Ar-**H**), 7.67–7.57 (m, 2H, Ar-**H**), 7.28–7.18 (m, 4H, Ar-**H**), 7.18–7.12 (m, 3H, Ar-**H**), 5.51 (s, 2H, Ar-C$\mathbf{H_2}$), 2.26 (s, 3H, Ar-C$\mathbf{H_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.6, 163.0, 142.5, 137.4, 136.9, 134.7, 134.6, 132.7, 131.7, 129.7, 127.7, 126.3, 123.1, 122.4, 121.9, 121.2, 120.4, 116.0, 111.7, 111.2, 49.9, 21.1; HRMS Calculated for $\mathbf{C_{24}H_{20}N_2O_3}$ [M-H]$^-$ 383.1396, found 383.1394.

2-(1-(4-(trifluoromethyl)benzyl)-1 H **-indole-3-carboxamido)benzoic acid (7o)**

Target compound **7o** were synthesized according to the synthetic procedure given above. white solid, yielding 46%. m.p. 231.0–232.6 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.70 (s, 1H, Ar-COO**H**), 11.83 (s, 1H, CON**H**), 8.75 (d, J = 8.3 Hz, 1H, Ar-**H**), 8.30 (s, 1H, N-C**H**=), 8.28–8.24 (m, 1H, Ar-**H**), 8.05 (dd, J = 7.9, 1.4 Hz, 1H, Ar-**H**), 7.72 (d, J = 8.2 Hz, 2H, Ar-**H**), 7.68–7.61 (m, 1H, Ar-**H**), 7.58–7.55 (m, 1H, Ar-**H**), 7.46 (d, J = 8.1 Hz, 2H, Ar-**H**), 7.28–7.22 (m, 2H, Ar-**H**), 7.16 (t, J = 7.6 Hz, 1H, Ar-**H**), 5.72 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.6, 162.9, 142.6, 142.4, 136.9, 134.7, 133.0, 131.7, 128.7(J_{C-F} = 31.6 Hz), 128.2, 126.3, 126.1(J_{C-F} = 3.8 Hz), 124.6(J_{C-F} = 270.5 Hz), 123.3, 122.5, 122.1, 121.3, 120.4, 116.1, 111.7, 111.6, 49.5; HRMS Calculated for $\mathbf{C_{24}H_{17}F_3N_2O_3}$ [M-H]$^-$ 437.1113, found 437.1091.

2-(5-chloro-1-(4-fluorobenzyl)-1 H -indole-3-carboxamido)benzoic acid (7p)

Target compound **7p** were synthesized according to the synthetic procedure given above. white solid, yielding 38%. m.p. 231.7–233.2 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.73 (s, 1H, Ar-COO**H**), 11.80 (s, 1H, CON**H**), 8.69 (d, J = 7.9 Hz, 1H, Ar-**H**), 8.30 (s, 1H, N-C**H**=), 8.23 (d, J = 2.0 Hz, 1H, Ar-**H**), 8.05 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.67–7.62 (m, 2H, Ar-**H**), 7.38–7.35 (m, 2H, Ar-**H**), 7.29 (dd, J = 8.8, 2.1 Hz, 1H, Ar-**H**), 7.23–7.12 (m, 3H, Ar-**H**), 5.58 (s, 2H, Ar-C$\mathbf{H_2}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 162.5, 162.1(J_{C-F} = 242.1 Hz), 142.2, 135.4, 134.7, 133.7, 133.6(J_{C-F} = 3.0 Hz), 131.7, 129.9(J_{C-F} = 8.1 Hz), 127.5, 126.9, 123.3, 122.6, 120.5, 116.2, 116.1(J_{C-F} = 21.7 Hz), 113.4, 111.0, 49.5; HRMS Calculated for $\mathbf{C_{23}H_{16}ClFN_2O_3}$ [M-H]$^-$ 421.0755, found 421.0745.

2-(5-chloro-1-(4-methoxybenzyl)-1 H -indole-3-carboxamido)benzoic acid (7q)

Target compound **7q** were synthesized according to the synthetic procedure given above. white solid, yielding 40%. m.p. 227.8–229.5 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.73 (s, 1H, Ar-COO**H**), 11.80 (s, 1H, CON**H**), 8.69 (d, J = 7.9 Hz, 1H, Ar-**H**), 8.27 (s, 1H, N-C**H**=), 8.22 (d, J = 2.0 Hz, 1H, Ar-**H**), 8.05 (dd, J = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.70–7.61 (m, 2H, Ar-**H**), 7.28 (dd, J = 8.7, 1.9 Hz, 3H, Ar-**H**), 7.19–7.12 (m, 1H, Ar-**H**), 6.91 (d, J = 8.7 Hz, 2H, Ar-**H**), 5.49 (s, 2H, Ar-C$\mathbf{H_2}$), 3.72 (s, 3H, Ar-O$\mathbf{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 162.5, 159.3, 142.2, 135.4, 134.7, 133.6, 131.7, 129.3, 129.2, 127.5, 126.8, 123.2, 122.6, 120.4, 120.4, 116.2, 114.6, 113.5, 110.7, 55.5, 49.8; HRMS Calculated for $\mathbf{C_{24}H_{19}ClN_2O_4}$ [M-H]$^-$ 433.0955, found 433.0946.

2-(5-chloro-1-(4-methylbenzyl)-1 H -indole-3-carboxamido)benzoic acid (7r)

Target compound **7r** were synthesized according to the synthetic procedure given above. white solid, yielding 46%. m.p. 187.4–229.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.47 (s, 1H, Ar-COO**H**), 8.64 (d, J = 8.0 Hz, 1H, Ar-**H**), 8.32 (d, J = 1.9 Hz, 1H, Ar-**H**), 8.24 (s, 1H, N-C**H**=), 8.03 (dd, J = 7.7, 1.5 Hz, 1H, Ar-**H**), 7.57 (d, J = 8.8 Hz, 1H, Ar-**H**), 7.39–7.32 (m, 1H, Ar-**H**), 7.23 (dd, J = 8.8, 2.0 Hz, 1H, Ar-**H**), 7.18–7.09 (m, 4H, Ar-**H**), 6.97 (t, J = 7.4 Hz, 1H, Ar-**H**), 5.51 (s, 2H, Ar-C$\mathbf{H_2}$), 2.24 (s, 3H, Ar-C$\mathbf{H_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 162.5, 141.9, 137.4, 135.3, 134.5, 133.1, 131.8, 131.4, 129.7, 128.1, 127.5, 126.5, 123.1, 122.9,

121.5, 120.9, 119.0, 113.2, 111.5, 50.0, 21.1; HRMS Calculated for **$C_{24}H_{19}ClN_2O_3$** [M-H]$^-$ 417.1006, found 417.1004.

2-(5-chloro-1-(4-(trifluoromethyl)benzyl)-1 H -indole-3-carboxamido)benzoic acid (7s)

Target compound **7s** were synthesized according to the synthetic procedure given above. white solid, yielding 36%. m.p. 245.2–247.0 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 1H, Ar-COO**H**), 11.84 (s, 1H, CON**H**), 8.70 (d, *J* = 8.3 Hz, 1H, Ar-**H**), 8.36 (s, 1H, N-C**H**=), 8.25 (d, *J* = 2.1 Hz, 1H, Ar-**H**), 8.05 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-**H**), 7.72 (d, *J* = 8.2 Hz, 2H, Ar-**H**), 7.67–7.60 (m, 2H, Ar-**H**), 7.45 (d, *J* = 8.1 Hz, 2H, Ar-**H**), 7.29 (dd, *J* = 8.8, 2.0 Hz, 1H, Ar-**H**), 7.18 (t, *J* = 7.2 Hz, 1H, Ar-**H**), 5.73 (s, 2H, Ar-C**H_2**).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 162.5, 142.3, 142.1, 135.4, 134.6, 134.0, 131.7, 128.8(J_{C-F} = 31.8 Hz), 128.2, 127.5, 127.0, 126.2(J_{C-F} = 3.9 Hz), 124.6(J_{C-F} = 270.5 Hz), 123.4, 122.7, 120.5, 120.4, 116.5, 113.5, 111.2, 49.7; HRMS Calculated for **$C_{24}H_{16}ClF_3N_2O_3$** [M-H]$^-$ 471.0723, found 471.0711.

2-(1-(4-fluorobenzyl)-1 H -indazole-3-carboxamido)benzoic acid (7t)

Target compound **7t** were synthesized according to the synthetic procedure given above. orange solid, yielding 50%. m.p. 223.3–224.8 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.70 (s, 1H, Ar-COO**H**), 12.64 (s, 1H, CON**H**), 8.86 (dd, *J* = 8.4, 0.8 Hz, 1H, Ar-**H**), 8.27 (d, *J* = 8.1 Hz, 1**H**, Ar-**H**), 8.08 (dd, *J* = 7.9, 1.6 Hz, 1H, Ar-**H**), 7.93 (d, *J* = 8.6 Hz, 1H, Ar-**H**), 7.71–7.63 (m, 1H, Ar-**H**), 7.56–7.43 (m, 3H, Ar-**H**), 7.40–7.33 (m, 1H, Ar-**H**), 7.25–7.13 (m, 3H, Ar-**H**), 5.80 (s, 2H, Ar-C**H_2**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 162.2(J_{C-F} = 242.7 Hz), 160.9, 141.4, 141.3, 137.8, 134.8, 133.2(J_{C-F} = 2.9 Hz), 131.8, 130.4(J_{C-F} = 8.6 Hz), 127.7, 123.7, 123.1, 122.9, 122.1, 120.1, 116.6, 116.0(J_{C-F} = 21.2 Hz), 111.3, 52.3; HRMS Calculated for **$C_{22}H_{16}FN_3O_3$** [M-H]$^-$ 388.1097, found 388.1091.

2-(1-(4-methoxybenzyl)-1 H -indazole-3-carboxamido)benzoic acid (7u)

Target compound **7u** were synthesized according to the synthetic procedure given above. Light yellow solid, yielding 44%. m.p. 254.9–256.1 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.73 (s, 1H, Ar-COO**H**), 12.66 (s, 1H, CON**H**), 8.87 (d, *J* = 8.3 Hz, 1H, Ar-**H**), 8.26 (d, *J* = 8.1 Hz, 1H, Ar-**H**), 8.08 (dd, *J* = 8.0, 1.2 Hz, 1H, Ar-**H**), 7.91 (d, *J* = 8.5 Hz, 1H, Ar-**H**), 7.68 (t, *J* = 7.3 Hz, 1H, Ar-**H**), 7.51 (t, *J* = 7.6 Hz, 1H, Ar-**H**), 7.42–7.30 (m, 3H, Ar-**H**), 7.21 (t, *J* = 7.5 Hz, 1H, Ar-**H**), 6.90 (d, *J* = 8.6 Hz, 2H, Ar-**H**), 5.72 (s, 2H, Ar-C**H_2**), 3.71 (s, 3H, Ar-OC**H_3**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 161.0, 159.4, 141.4, 141.1, 137.5, 134.8, 131.8, 129.7, 128.9, 127.5, 123.6, 123.1, 123.0, 122.1, 120.1, 116.5, 114.5, 111.4, 55.5, 52.7; HRMS Calculated for **$C_{23}H_{19}N_3O_4$** [M-H]$^-$ 400.1297, found 400.1296.

2-(1-(4-methylbenzyl)-1 H -indazole-3-carboxamido)benzoic acid (7v)

Target compound **7v** were synthesized according to the synthetic procedure given above. orange solid, yielding 46%. m.p. 264.6–265.3 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 1H, Ar-COO**H**), 12.66 (s, 1H,

CONH), 8.87 (d, *J* = 7.7 Hz, 1H, Ar-H), 8.27 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.08 (dd, *J* = 7.9, 1.6 Hz, 1H, Ar-H), 7.89 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.71–7.64 (m, 1H, Ar-H), 7.53–7.47 (m, 1H, Ar-H), 7.35 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.30 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.23–7.18 (m, 1H, Ar-H), 7.15 (d, *J* = 7.9 Hz, 2H, Ar-H), 5.74 (s, 2H, Ar-CH_2), 2.25 (s, 3H, Ar-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 161.0, 141.4, 141.2, 137.7, 137.6, 134.7, 134.0, 131.8, 129.7, 128.2, 127.6, 123.6, 123.1, 123.0, 122.1, 120.1, 116.6, 111.4, 53.0, 21.1; HRMS Calculated for $C_{23}H_{19}N_3O_3$ [M-H]$^-$ 384.1348, found 384.1340.

2-(1-(4-(trifluoromethyl)benzyl)-1 H **-indazole-3-carboxamido)benzoic acid (7w)**

Target compound **7w** were synthesized according to the synthetic procedure given above. orange solid, yielding 41%. m.p. 224.5–226.0 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (s, 1H, CONH), 8.85 (dd, *J* = 8.4, 0.8 Hz, 1H, Ar-H), 8.29 (d, *J* = 8.2 Hz, 1H, Ar-H), 8.07 (dd, *J* = 7.9, 1.6 Hz, 1H, Ar-H), 7.91 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.73 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.71–7.65 (m, 1H, Ar-H), 7.59–7.50 (m, 3H, Ar-H), 7.41–7.35 (m, 1H, Ar-H), 7.24–7.18 (m, 1H, Ar-H), 5.93 (s, 2H, Ar-CH_2);^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 160.8, 141.7, 141.5, 141.3, 138.1, 134.7, 131.8, 128.9(J_{C-F} = 31.8 Hz), 128.7, 127.9, 126.1(J_{C-F} = 3.6 Hz), 124.6(J_{C-F} = 270.4 Hz), 123.8, 123.1, 122.9, 122.2, 120.2, 116.6, 111.2, 52.4. HRMS Calculated for $C_{23}H_{16}F_3N_3O_3$ [M-H]$^-$ 438.1066, found 438.1049.

4.2 Biological activity

4.2.1 *In vivo* anti-inflammatory activity

Materials required: male ICR mice model weighing 18–24 g; Xylene; Indomethacin; Sodium carboxymethyl cellulose solution (CMC-Na) were provided by Hefei Yigong Medicine Co., Ltd.

Experiment: Animals were randomly divided into 25 groups according to their respective weights, 10 were in each group. The groups were divided into the model group, the positive group, and 23 others as compound groups. The positive group was administered with indomethacin 10 mg/kg, and the compound groups were given compound 5 mg/kg, respectively. All drugs were injected by intragastric administration. The drug was administered once a day for 6 days. After 7 days of adaptive feeding of each group, the right ear of the mice was coated with xylene 20 μL uniformly, and for comparison, the left ear was layered with a solution of 0.5% CMC-Na of the same amount as xylene. After 1 h, the animals were sacrificed, and a hole puncher of 8 mm was used to slice round ears from the same sites of left and right ears and weighed to calculate the swelling degree and inhibition rate. The calculation was carried out according to the following equations [28].

Swelling degree (mg) = VR- VL.

Inhibition (%) = ((VR- VL) control - (VR- VL) treated)/(VR- VL) control × 100%.

VR = Average weight of the right ear; VL = Average weight of the left ear.

4.2.2 *In vitro* COX-1 and COX-2 inhibitory assay

The ability of the active carboxylic acid derivatives **7f** and **7n** and reference drugs taken such as celecoxib, indomethacin, zileuton, and mefenamic acid to inhibit human COX-1 and COX-2 enzymes (IC_{50} value, nM) were determined. The inhibitory activity of the tested compounds and the reference drugs were assayed using a COX-1 inhibitor fluorometric screening kit (BioVision, Inc., Mountain View, CA, USA) and a COX-2 inhibitor screening kit (Beyotime Biotech Co., Ltd, China), according to the manufacturer's instructions. The assay buffer for COX-1/COX-2 (398 mL), COX-1 cofactor (2 mL), and arachidonic acid solution were added to a 96-well plate. The test compounds were then added to the above solution in respective wells. The 96-well plate was incubated for 5 min at 37°C, and fluorescence was measured. The excitation and emission wavelengths were 535 nm and 587 nm, respectively.

The groups were divided into RFU (Relative Fluorescence Unit) blank control, RFU 100% enzyme activity control, RFU positive drug control, and RFU test compound for depicting different results. The inhibition rate was calculated according to the following equations.

Inhibition rate (%) = (RFU (enzyme)-RFU (test compound/positive))/(RFU (enzyme)-RFU (blank) × 100%

RFU (enzyme) = RFU 100% enzyme activity control; RFU (test compound) = RFU test compound; RFU (blank) = RFU blank control; RFU (positive) = RFU positive drug control

The assays were reproduced in triplicates, and the IC_{50} values were calculated from the concentration curves [28].

4.2.3 *In vitro* 5-LOX inhibitory assay

The inhibitory assay against 5-LOX enzyme was conducted for compounds **7f** and **7n** as well as zileuton and mefenamic acid using lipoxygenase inhibitor screening assay supplied by Cayman chemicals. The procedure was conducted according to the instructions given in the assay kit in accordance with the previously reported methods to evaluate the IC_{50} values of the tested compounds [48].

4.2.4 Assay for NO and PGE2 inhibition

RAW264.7 cells were cultured in DMEM medium containing penicillin (final concentration 100 U/mL), streptomycin (final concentration 100 μg/mL), and 10% FBS. When the cells reached 90% confluency, the medium was collected and centrifuged at 1000 rpm for 5 min. The supernatant was aspirated, and the medium was added again, resuspending the cells while washing the remaining adherent cells twice with 2 mL of phosphate buffer saline (PBS). The PBS solution was discarded 2 mL of 0.25% (w/v) Trypsin-0.53 mM EDTA mixed digestion solution was added and observed under a microscope for about 60 s. When the cells became round, 2 mL of complete medium was added quickly to terminate the digestion and pipetted to collect the cells gently. It was centrifuged at 1000 rpm for 5 min, the supernatant was discarded, and the cells were resuspended in a culture medium; the suspended cells were mixed and recovered in separate culture bottles with media being changed daily.

PGE_2 levels were detected using ELISA: (1) 50 µL sample or standard was added to each well. The standard concentrations are found to be 24.69, 74.07, 222.22, 666.67, 2000 pg/mL. (2) Afterward, 50 µL of the prepared Detection Reagent A was added and mixed well and incubated at 37°C for 1 h. (3) Then, it was washed 3 times. (4) 100 µL of the prepared Detection Reagent B was added and incubated at 37°C for 30 min. (5) It was washed 5 times. (6) 90 µL of Substrate Solution was then added and incubated at 37°C for 10–20 min. (7) 50 µL of Stop Solution was then added to stop the reaction. Immediately a microplate reader was used to determine the OD value at 450 nm.

Griess method to observe NO level: (1) The Griess Reagents I and II were taken and allowed to return to room temperature. (2) The standard was diluted with the solution used for testing the sample. The concentration of the standard is found to be 0, 1, 2, 5, 10, 20, 40 ,60, 80, 100 µM. (3) 50 µL of standard or sample was then added to each well. (4) 50 µL/well was pressed, and Griess Reagent I was added at room temperature to each well. (5) 50 µL/well was pressed, and Griess Reagent II was added at room temperature to each well. (6) The OD value was measured at 450 nm with a microplate reader [49, 50].

4.2.5 *In vitro* cytotoxic activity

RAW264.7 cell line was bought from the American Tissue Culture Collection (ATCC, Manassas, VA, USA).

The tested cell lines were suspended (ca. 1.0 × 105 cells/mL) in 96-well microtiter plates, and incubations were done with a serum-free medium for 2 h at 37°C and 5% CO_2. Subsequently, the media was discarded and again incubated with different concentrations of compounds (concentration: 0 mM, 5 mM, 10 mM, 20 mM, 30 mM) with RPMI 1640 medium for 24 h. Four hours prior to incubation, 20 mL of MTT solution (5 mg/mL) was added to each well. After the 4 h incubation, the 96-well plate was centrifuged at 1500 rpm for 3 min, the supernatants were discarded individually, and 150 mL of dimethyl sulfoxide (DMSO) was added to each well, and the plate was shaken on a shaker for 10 min till the crystals dissolved sufficiently. The OD at 570 nm was measured by a micro-plate reader [51].

4.2.6 Molecular docking study

The molecular docking simulation was performed using ANTODOCK 4.2.6 software embedded into AutoDockTools-1.5.6. The active sites were generated from the co-crystallized ligands (IMM and SC-558) within COX-1 and COX-2 protein structures (PDB codes: 1PGF and 1CX2), respectively. Polar hydrogen was added to all ligands and proteins with the AutoDock Tools (ADT) program before docking with AutoDock 4.2.6. All graphical representations in Figs. 5–6 were rendered using the PyMOL tool [41–44].

4.3.7 Statistical analysis

Results are expressed as mean standard deviation (SD) and were analysis statistically with analysis of variance (ANOVE), and the Tukey method were assessed differences between groups. A value of $p < 0.05$ is considered to be statistically significant.

Declarations

Supporting Information The ^{1}H NMR, ^{13}C NMR, ESI-MS, and HRMS spectra of the target compounds.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests

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Scheme

Scheme 1 is available in supplementary section.

Figures

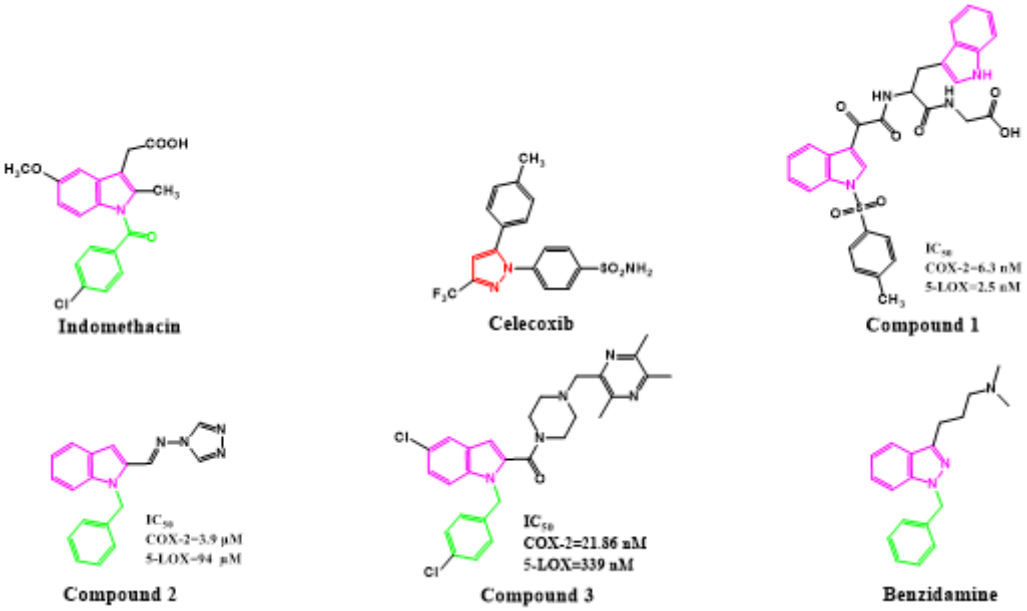

Figure 1

Chemical structures of representative dual COX/LOX compounds

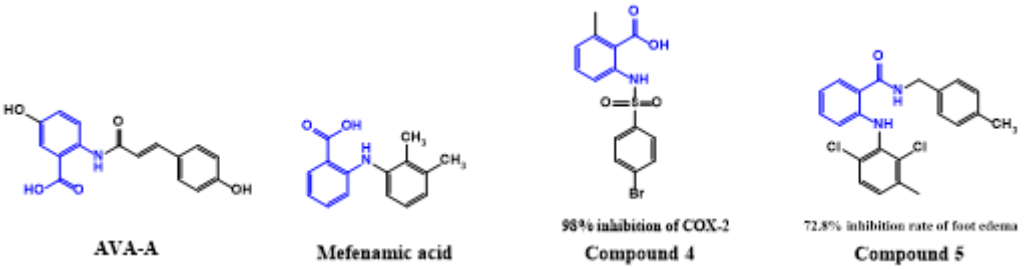

Figure 2

N-arylanthranilic acid derivatives with anti-inflammatory activity

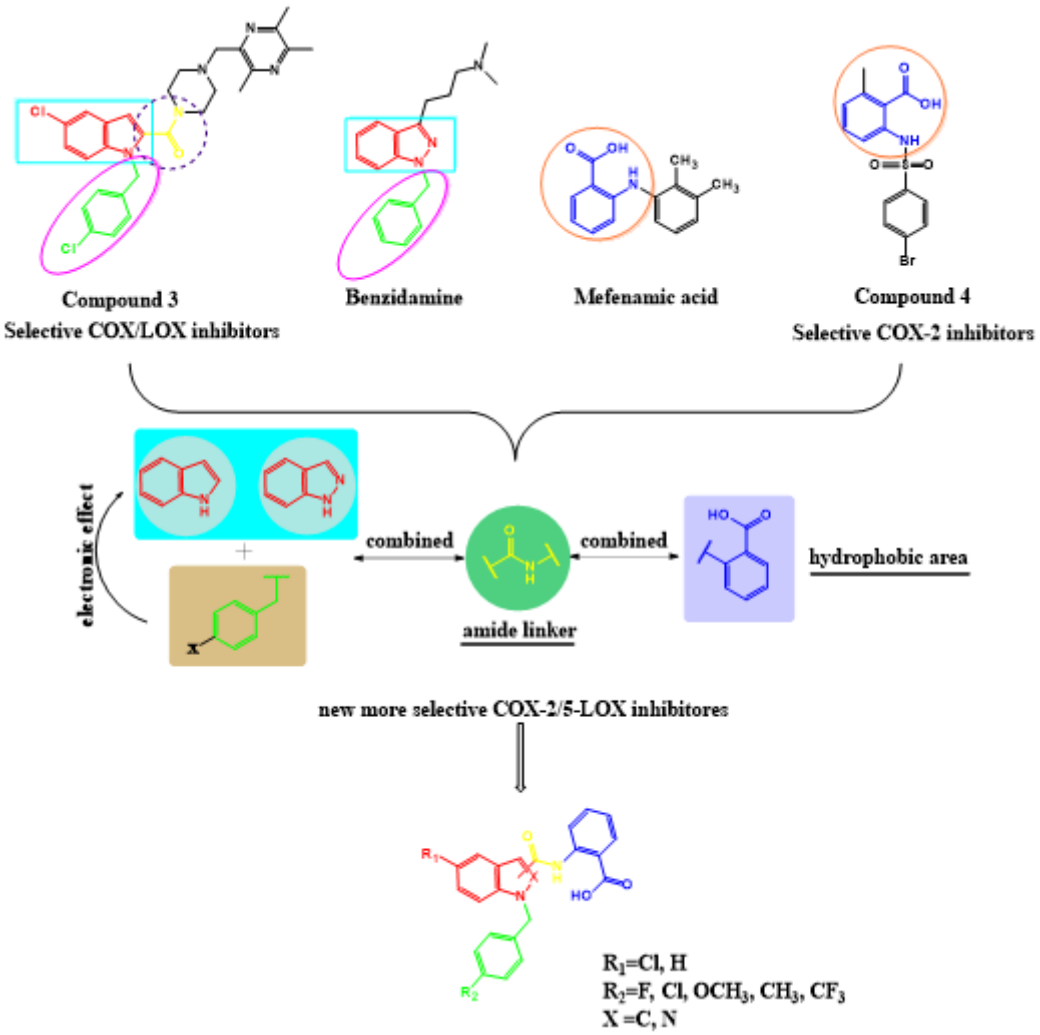

Figure 3

Idea of novel 2- (indole arylamide) benzoic acid analogs

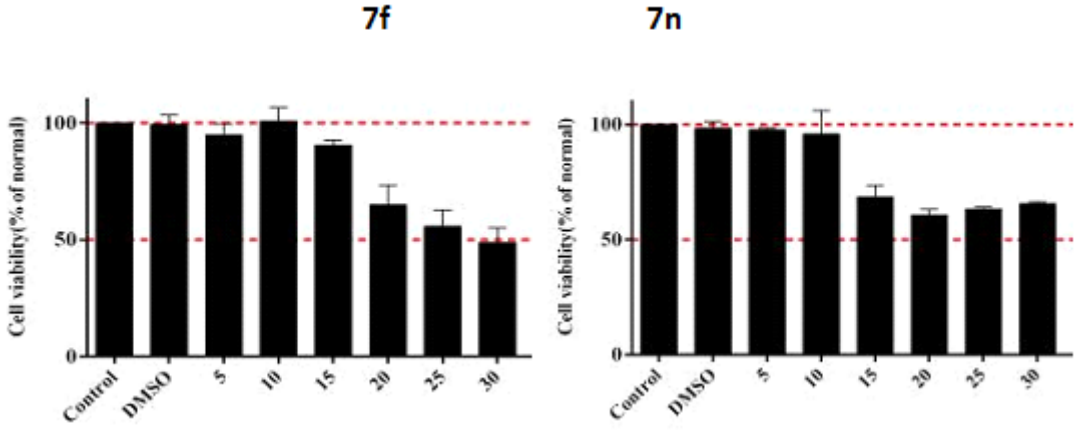

Figure 4

Cell viability experiment was performed at 5-30 μM concentration

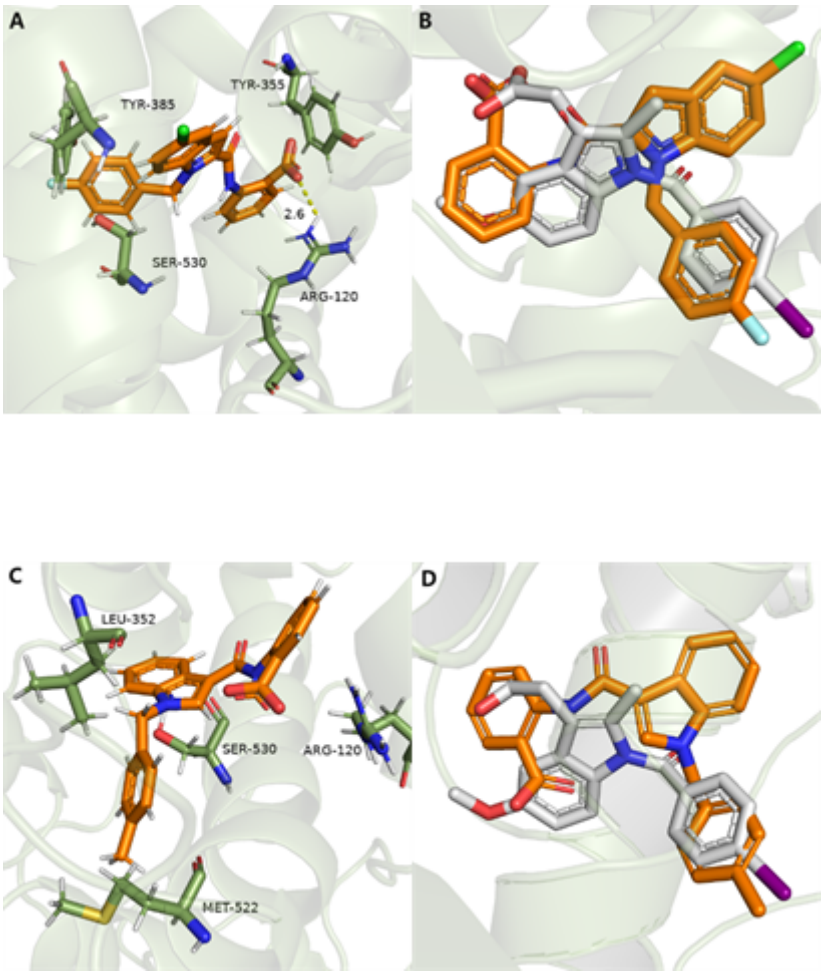

Figure 5

(A) Docking and binding pattern of compound **7f** (orange) into COX-1 active site (PDB code: 1PGF). (B) The superimposition of the docked pose **7f** (orange) and the co-crystallized IMM (grey) within active site of COX-1. (C) Docking and binding pattern of compound **7n** (orange) into COX-1 active site (PDB code: 1PGF). (D) The superimposition of the docked pose **7n** (orange) and the co-crystallized IMM (grey) within active site of COX-1. Dashed lines represent hydrogen bonds.

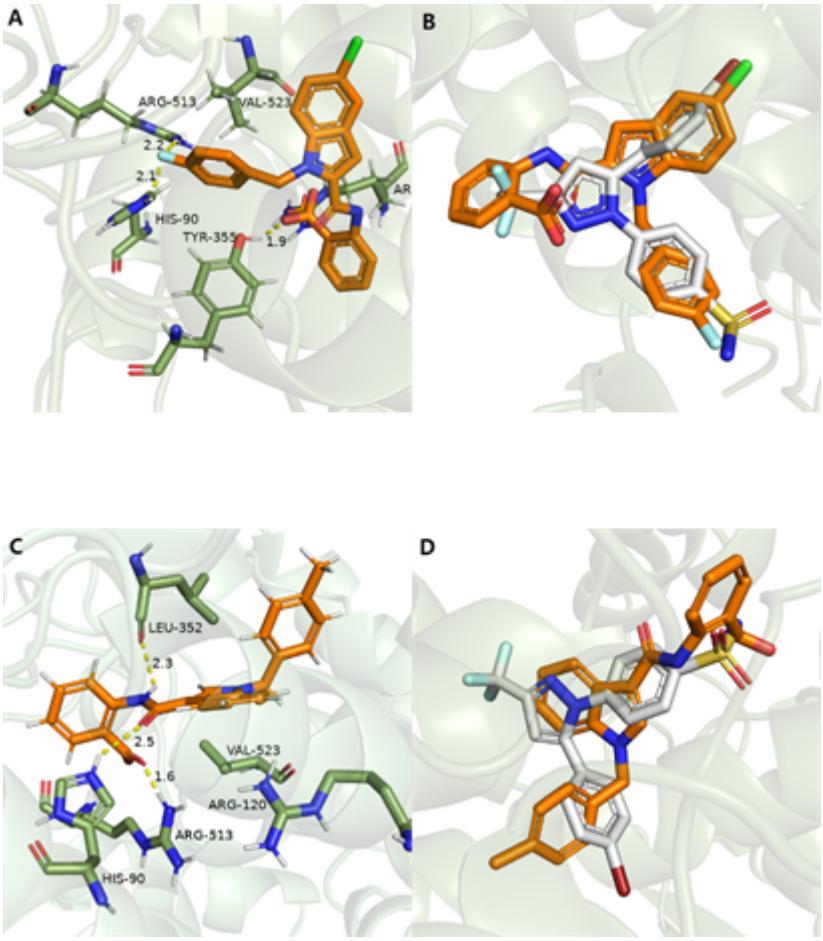

Figure 6

(A) Docking and binding pattern of compound **7f** (orange) into COX-2 active site (PDB code: 1CX2). (B) The superimposition of the docked pose **7f** (orange) and the co-crystallized IMM (grey) within active site of COX-2. (C) Docking and binding pattern of compound **7n** (orange) into COX-2 active site (PDB code: 1CX2). (D) The superimposition of the docked pose **7n** (orange) and the co-crystallized IMM (grey) within active site of COX-2. Dashed lines represent hydrogen bonds.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.png
- SupplementaryMaterial.docx