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Received: 18 April 2023 | Revised: 19 September 2023 | Accepted: 20 September 2023

DOI: 10.1002/ardp.202300217

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Archiv der Pharmazie

FULL PAPER

5-Fluoro/(trifluoromethoxy)-2-indolinone derivatives with anti-interleukin-1 activity

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Funding information
Scientific and Technological Research Council of Turkey (TUBITAK), Grant/Award Number: 1003-215S011

Abstract

The pro-inflammatory cytokine interleukin-1 (IL-1) drives the pathogenesis of several inflammatory diseases. Recent studies have revealed that 2-indolinones can modulate cytokine responses. Therefore, we screened several 2-indolinone derivatives in preliminary studies to develop agents with anti-IL-1 activity. First, the putative efficacies and binding interactions of 2-indolinones were evaluated by docking studies. Second, previously synthesized 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **47–69**) which had the highest inhibitory effect in the screening were evaluated for inhibitory effects on the IL-1 receptor (IL-1R). Compounds **52** (IC_{50} = 0.09 μM) and **65** (IC_{50} = 0.07 μM) were selected as lead compounds for the subsequent synthesis of new derivatives. The novel 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **70–116**) were designed, synthesized, and in vitro studies were completed. The compounds **76**, **78**, **81**, **91**, **100**, **105**, and **107** tested showed nontoxic inhibitory effects on IL-1R-dependent responses in the range of 0.01–0.06 μM and stronger than the lead compounds **52** and **65**. In vitro and in silico findings showed that compounds **78** (IC_{50} = 0.01 μM) and **81** (IC_{50} = 0.02 μM) had the strongest IL-1R inhibitory effects and the most favorable drug-like properties. Molecular modeling studies of the compounds

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78 and **81** were carried out to determine the possible binding interactions at the active site of the IL-1R.

KEYWORDS
biological activity, cytokines, molecular modeling, structure–activity relationships, synthesis design

1 | INTRODUCTION

Since the first observations that a soluble factor of monocyte-macrophage origin induces the release of PGE2 from synovial tissue cultures, extensive research has focused on ever-growing interleukin-1 (IL-1) family-mediated responses.[1] Activated by the cytokine IL-1, this acute immune response plays a critical role in several autoimmune diseases such as Behcet's disease, gout, adult-onset Still's disease, and familial Mediterranean fever (FMF). It has also been associated with rheumatoid arthritis, albeit remotely, in other complex chronic diseases such as type 2 diabetes, cancer, and Alzheimer's.[2–4] Recently, there has been a renewed interest in blocking IL-1 responses to prevent COVID-19 pneumonia-related hyper inflammation syndrome as the virus induces monocyte-derived macrophages to release high amounts of IL-1β and several other cytokines.[5,6] Overall, IL-1 activation serves as a fast signal to trigger inflammatory responses, converging essential mediators such as TNF-α, IL-18, and IL-6, thereby augmenting different inflammatory and metabolical responses.[7] IL-1 binds the cell surface receptor IL-1R1 linked to the intracellular TIR domain, similar to toll-like receptors (TLRs). IL-1R1 requires the so-called IL-1R accessory protein or IL-1R3 to form a complex for initiating the response.[8] Once activated, IL-1R1 sends the message downstream, resulting in hyperthermic and inflammatory effects mediated by the release of PGE2 and TNF-α. Since IL-1R1 activation is required to induce and maintain several different immune system-mediated responses, its activity is tightly regulated by redundant mechanisms. Namely, an endogenous receptor antagonist, IL-1Ra, competes with IL-1 for its binding site, thereby suppressing the receptor activation. Additionally, a decoy receptor, named IL-1R2, binds IL-1, but its TIR domain does not activate the acute immune response due to missing catalytic properties. These regulatory mechanisms provide a fine-tuning of the responses to IL-1 by changing the composition of the IL-1 response elements.[9]

Clinically, there are several different approaches used to block IL-1-mediated activity. IL-1R1 activation can be inhibited with synthetic IL-1Ra (anakinra), strengthening endogenous suppressive activity. Secreted IL-1 can also be blocked by either neutralizing antibodies (canakinumab) or recombinant decoy receptor (rilonacept) thus, weakening the IL-1 effects. Previously, attempts were made to develop peptide entities to block the IL-1 receptor.[10–12] However, only isunakinra, an IL-1β/IL-1Ra chimeric peptide, has progressed into clinical trials.[13] Small molecules offer an important cost advantage over biological drugs, and they can be more suitable for patients owing to more versatile pharmaceutical forms with better compliance. Thus, developing a safe and effective molecule capable of controlling the effects of IL-1 is of prime importance.[14,15]

Indole, first isolated in 1866, is a significant ring system found in the structure of various natural products, and on which many recent studies have been carried out due to its pharmaceutical and pharmacological importance. It is the precursor of many active pharmaceutical ingredients such as cyclooxygenase (COX) inhibitors, indomethacin, acemethazine, and etodolac. Tenidap (Pfizer), which carries a 2-indolinone ring is a cytokine modulator and COX/5-lipoxygenase (5-LOX) inhibitor[16] (Figure 1). Tenidap and other 3-substituted-2-oxindole-1-carboxamides were first synthesized in 1985 and patented as both COX and 5-LOX enzyme inhibitors.[17] Subsequent studies have shown that in addition to COX and 5-LOX inhibitions, it also inhibits the lipopolysaccharide (LPS)-induced synthesis of IL-1 and IL-6 in macrophages.[18,19] Another indole derivative MK-886 (Sandoz) is a selective inhibitor of 5-lipoxygenase-activating protein (FLAP) that potently suppresses leukotriene biosynthesis (Figure 1).[20,21] In a study examining the effects of some 5-LOX inhibitors on the modulations of IL-1, IL-6, and IL-8 cytokines, it was found that MK-886 is a strong IL-1 synthesis inhibitor. It was determined that the other two cytokines inhibited synovial production to a lesser extent.[22]

Based on these findings, research focused on the replacement of the thiophene ring has been carried out to develop new anti-inflammatory agents with the 3-substituted 2-indolinone structure. Alkyl, cycloalkyl, cycloalkenyl, phenyl, substituted phenyl, naphthyl, heterocycly, or heteroaryl moieties were replaced by the thiophene ring on tenidap. Generally, the inhibitory activities of 2-indolinone derivatives developed with these modifications increased and their toxicity decreased. Moreover, the contribution of groups at position 5 or 6 of the 2-indolinone ring to the inhibition was investigated. The

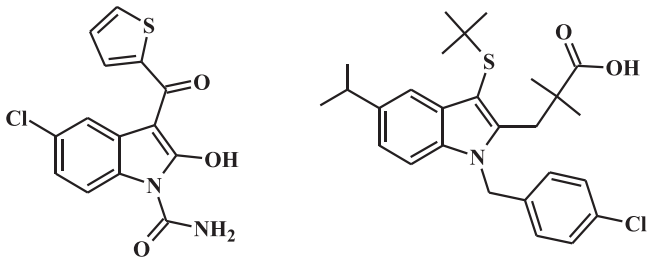


FIGURE 1 Anti-inflammatory drugs bearing an indole ring.

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structure–activity relationship (SAR) analysis showed that the electron withdrawing groups at position 5 of the 2-indolinone ring had the highest inhibitory activity and the importance of the 2-indolinone ring for anti-inflammatory effect.[23–27] It was found that 5-bromo/fluorine substituted 2-indolinone derivatives carrying a 4-methylsulfonylphenyl residue at the 3-position have higher COX and 5-LOX inhibitory effects and also COX-2 selectivity compared to tenidap.[28]

We investigated the IL-1R inhibitory activities using in silico and in vitro methods of 2-indolinone derivatives synthesized and published by our research group.[29–38] Since the 2-indolinone-containing compounds are important cytokine modulators, we obtained compelling SAR data for 2-indolinone products. In vitro tests have shown that most 2-indolinone derivatives have significant IL-IR inhibitory activity, provided that the thiosemicarbazone structure is maintained. We concluded that the compounds bearing 4-phenylthiosemicarbazone residues at position 3 of the 2-indolinone ring (**I**)[29–31,34–37] have the highest inhibitory activity. Replacing methyl, ethyl, allyl, *n*-butyl, cyclohexyl, and benzyl groups of the phenyl ring at the thiosemicarbazone moiety significantly reduced the activity.

Moreover, we determined that 3-(4-oxothiazolidine)hydrazones (**III**)[31–33] synthesized from 3-thiosemicarbazone derivatives, as well as 3-semicarbazone derivatives (**II**)[36,37] and 3-spiro-2-indolinones (**IV**)[38] showed inhibitory activity above 10 µM. The inhibitory effect was increased in the compounds carrying methyl, ethyl, or benzyl groups at position 1 of the indole ring. In contrast, the activity was decreased in 1-nonsubstituted derivatives or N-Mannich bases. The highest inhibitory effect was obtained in compounds with fluorine or trifluoromethoxy groups at position 5 of the indole ring (Figure 2).

In this study, IL-1R binding interactions in silico and anti-IL-1 effects in vitro of 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) were evaluated and the leader compounds were determined. The binding interactions and physico-chemical properties to predict drug-likeness of the novel compounds designed according to in silico and preliminary in vitro test results were investigated. Based on the in vitro test results, the contribution of substituents at positions 1 and 5 of the indole ring and at positions 3 or 4 of the 4-phenylthiosemicarbazone moiety were determined.

2 | RESULTS AND DISCUSSION

2.1 | Molecular modeling

To identify novel potential IL-1R inhibitors we initially screened in silico 2-indolinone derivatives that were previously reported[29–38] for IL-1R affinity via docking, and the in silico findings confirmed preliminary in vitro screening test results. The binding free energies (ΔG) and calculated binding affinity (QK_d) values of the screened compounds were presented in Table 1. The first striking finding was that the binding free energy was lower in cyclohexyl, benzyl, and especially

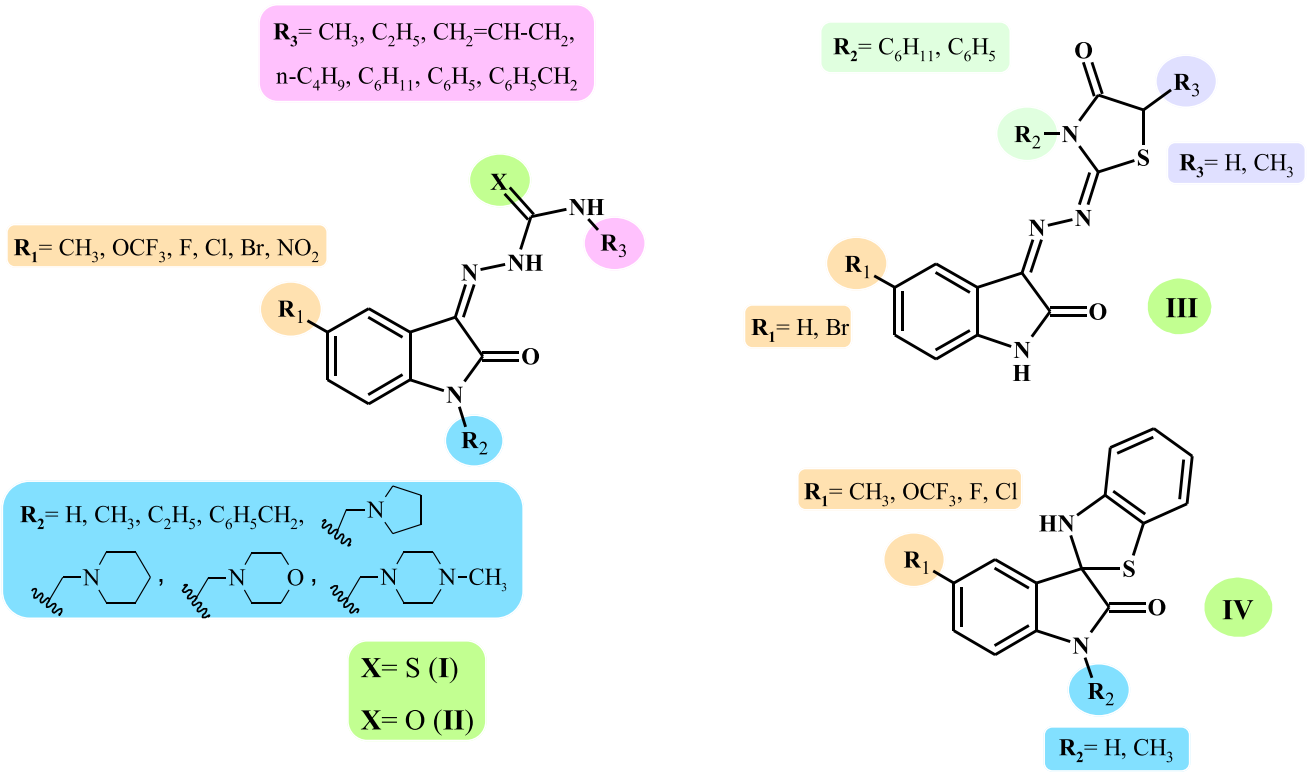
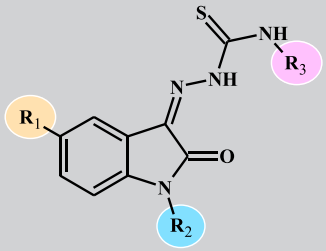


FIGURE 2 2-Indolinone derivatives tested for interleukin (IL)-1R inhibitory effect in preliminary studies. (**I**) Substituted 1*H*-indole-2,3-dione 3-thiosemicarbazones, (**II**) substituted 1*H*-indole-2,3-dione 3-semicarbazones, (**III**) 3-(3-substituted 4-thiazolidone-2-hydrazono)−2-indolinones, (**IV**) 3*H*-spiro[1,3-benzothiazole-2,3'-indol]−2'(1'*H*)-ones.

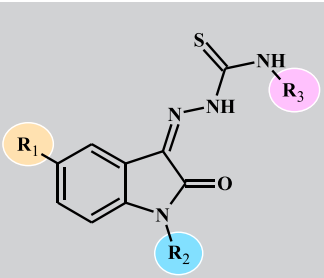
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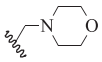
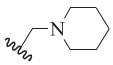
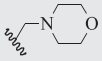
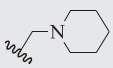
TABLE 1 The binding free energies (Δ*G*) and calculated K_d values (QK_d) of 2-indolinone derivatives screened for interleukin (IL)-1R inhibitory effect in in silico studies.



R_1	R_2	R_3	Δ*G* (kJ/mol)	QK_d (μM)
OCF_3	H	CH_3	−25.53	49.9
		C_2H_5	−25.67	47.3
		CH_2=CH-CH_2	−25.02	60.8
		n-C_4H_9	−27.46	23.6
		C_6H_{11}	−29.00	13.0
		C_6H_5	−30.05	8.64
		$C_6H_5CH_2$	−34.46	1.56
	CH_3	CH_3	−27.24	25.7
		C_2H_5	−25.79	45.1
		CH_2=CH-CH_2	−26.72	31.4
		n-C_4H_9	−26.18	38.8
		C_6H_{11}	−30.33	7.75
		C_6H_5	−30.89	6.24
		4-$CH_3C_6H_4$ (**65**)	−33.73	2.07
		$C_6H_5CH_2$	−30.89	6.24
	C_2H_5	C_6H_{11}	−30.69	6.74
		CH_3	−24.03	89.3
		C_2H_5	−28.07	18.6
		CH_2=CH-CH_2	−28.02	19.0
		n-C_4H_9	−26.03	41.1
		C_6H_{11}	−29.67	10.0
		C_6H_5	−29.72	9.82
		$C_6H_5CH_2$	−35.06	1.24
CH_3	H	C_6H_{11}	−29.78	9.59
		C_6H_5	−32.84	2.93
		$C_6H_5CH_2$	−35.29	1.13
	CH_3	C_6H_{11}	−30.64	6.87
		C_6H_5	−33.00	2.75
		$C_6H_5CH_2$	−30.84	6.36
F	H	CH_3	−26.28	37.3
		CH_2=CH-CH_2	−26.55	33.6

TABLE 1 (Continued)



R_1	R_2	R_3	Δ*G* (kJ/mol)	QK_d (μM)
		n-C_4H_9	−26.06	40.6
		C_6H_{11}	−29.03	12.8
		C_6H_5	−31.96	4.12
		$C_6H_5CH_2$	−34.62	1.47
	CH_3	3-ClC_6H_4 (**52**)	−32.55	3.27
		CH_3	−28.59	15.2
		C_2H_5	−29.49	10.7
		n-C_4H_9	−28.95	13.2
		C_6H_{11}	−31.81	4.36
		C_6H_5	−31.49	4.94
		$C_6H_5CH_2$	−32.10	3.90
		CH_3	−27.47	23.5
		C_2H_5	−28.76	14.2
		n-C_4H_9	−29.59	10.3
		C_6H_5	−34.92	1.30
		$C_6H_5CH_2$	−33.66	2.13
NO_2	H	C_2H_5	−32.06	3.96
		CH_2=CH-CH_2	−34.43	1.58
		C_6H_{11}	−29.89	9.19
		C_6H_5	−32.66	3.14
		CH_2=CH-CH_2	−31.73	4.50
		C_6H_{11}	−32.23	3.70
		C_6H_{11}	−34.56	1.50
		C_6H_5	−35.24	1.15

phenyl substituted compounds at the R_3 position compared to other derivatives. It was found that the 1-methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-methylphenyl)thiosemicarbazone] (compound **65**)[30] and 1-methyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(3-chlorophenyl) thiosemicarbazone] (compound **52**)[39] with mid-nanomolar activity (IC_{50} = 70 and 90 nM, respectively) on IL-1R inhibition displayed very low binding free energy (−33.73 kJ/mol, QK_d: 2.07 μM and −32.55 kJ/mol, QK_d: 3.27 μM, respectively) and have been selected as the lead compounds for subsequent synthesis of new derivatives.

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We then performed molecular dynamics simulation studies with compound **65** to establish the binding site at IL-1R. A 10 ns molecular dynamics for the system was performed. The complex with the protein and compound **65** solvated in a TIP3P water box and counter ions were added to neutralize the system. CHARMM36 force field was used to parametrize the atoms. Time step of simulations were kept as 2 fs. Initially, the energy of the system was minimized for 8000 steps and the system was gradually heated up to 310 K. The annealing step was followed by an equilibration period at constant temperature of 310 K and constant pressure of 1 bar for 10,000 steps. Finally, the molecular dynamics of the equilibrated system was performed for 5000 steps. Throughout the simulations, a small number of water molecules remained hydrogen bonded to **65**. Stationary water molecules were shown in the figure along with the hydrogen bonds. The binding region of compound **65** was similar to the binding sites of IL-1β and anakinra (Figure 3a). It consisted of the following amino acids Phe111, Lys112, Gln113, Val124, Cys125, Pro126, Tyr127, Met128, Glu129, Leu138, and Asp162. This finding underlined the importance of Lys112 for the binding of anakinra to IL-1R (Figures 3b,c and 4).[37,40] Figure 3c showed the best pose of the molecule in interaction with the residues of the protein (these residues were given in Figure 4).

2.1.1 | Analysis of docking results

When we examined all docking poses, it was found that the poses of the compounds which had 3/4-methoxy, 4-(trifluoromethoxy), 4-thiomethyl substituted compounds at R_3 substitution were largely

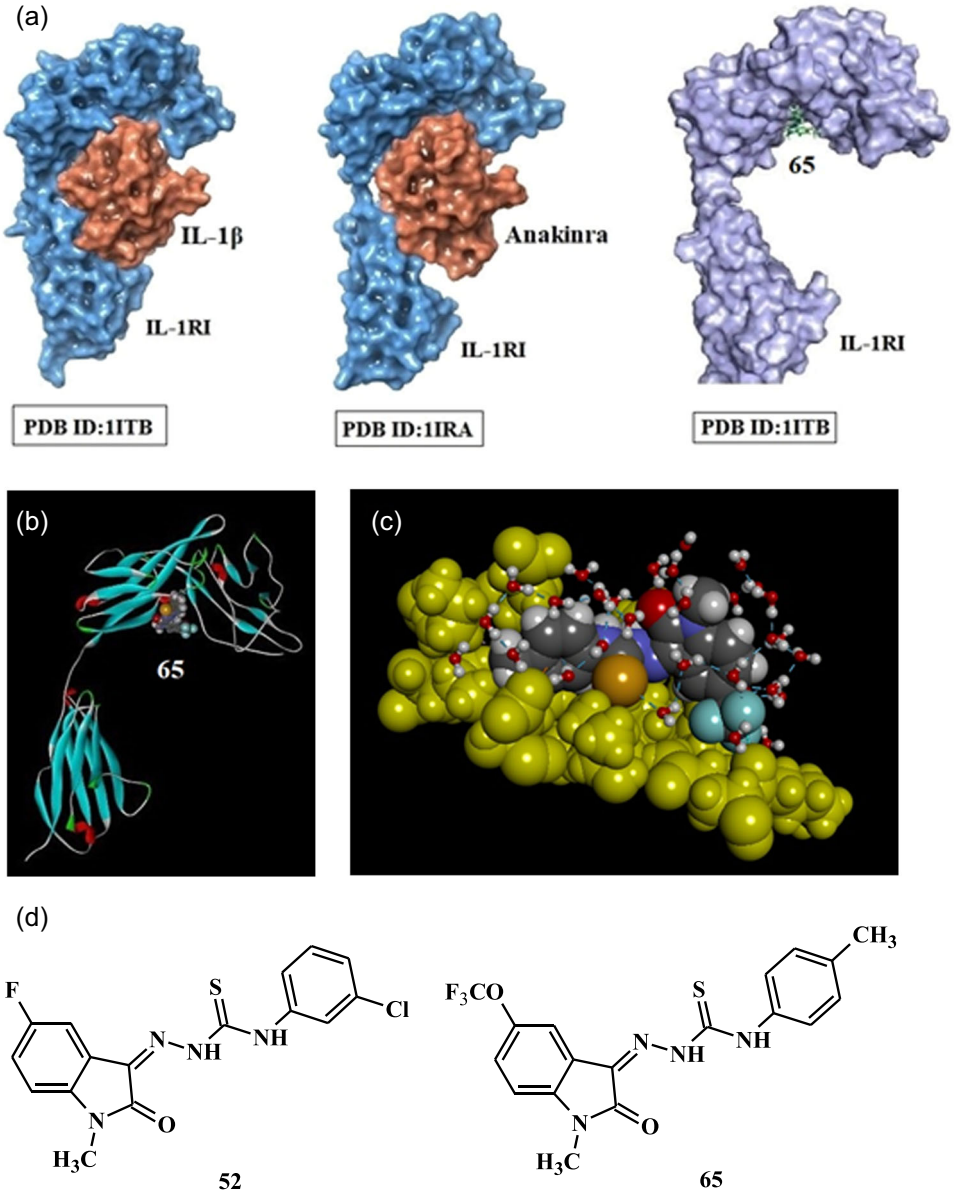
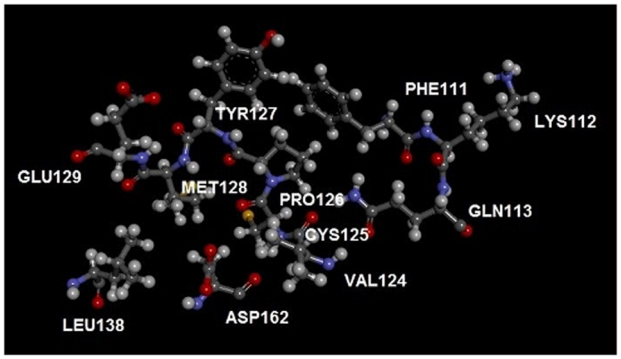


FIGURE 3 In the part of a, the crystal structure complexes of interleukin (IL)-1R1 with IL-1β, anakinra, and compound **65** appear as their surface areas stained with colorful, respectively. In the parts of b and c, best binding conformation of compound **65** and zoomed-in view of this conformation are seen. In part of d, the structures of compounds **52** and **65** are seen.

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Ligand atom	IL-1β residue and atom	Type of bonding
F1	Lys112;HN	Hydrogen bond
S	Cys125;O	Hydrogen bond
F1	Phe111;C	Halogen acceptor
F1	Lys112;O	Halogen acceptor
F2	Lys112;O	Halogen acceptor
N3	Tyr127	Electrostatic
Phe	Asp162;OD2	Electrostatic
Phe	Met128;HN	Pi-Hydrogen
Phe	Tyr127;Phe	Pi-Pi
Phe	Met128;C,O;Glu129;N	Amide Pi-Stacked
C18	Lys132	Hydrophobic
C18	Leu138	Hydrophobic

FIGURE 4 Interactions established by compound **65** with the interleukin (IL)-1R1 protein.

alike with the docking poses of the lead compounds **52** and **65**; whereas the compounds which had 3/4-(trifluoromethyl), 3-fluorine or chlorine substituted at R_3 were partially alike. This evaluation was also consistent with the in vitro test results. We observed that the compounds with no substitution at R_2 did not display promising activity. However, the compounds with benzyl moiety at R_2 substitution made it challenging to capture ligand-receptor interactions required for activity potentially due to the larger benzyl group compared to methyl or ethyl substitution.

Compound **78** formed two hydrogen bonds, the first between N_4 hydrogen and Ile110, and the second between the methoxy oxygen on the phenyl ring with Val16. In addition, hydrophobic interactions with Phe111, Lys112, and Lys114 amino acids of IL-1R1 were detected with the methyl group of the compound (Figure 5a,b). Similarly, compound **81** also displayed hydrogen bonds between N_4 hydrogen and Gln108, and between the lactam carbonyl oxygen of the indole ring with Lys112 (Figure 5c,d).

2.1.2 | Prediction of physicochemical properties

Compliance with *Lipinski's rule of five* (RO5)[41] and predicted pharmacokinetic properties of the designed and synthesized compounds were determined in silico and compared with the lead compounds **52** and **65** (Table 2). All compounds have molecular weights of less than 500 Daltons, and meet the molecular weight criteria of RO5. According to the criteria of RO5, the numbers of hydrogen bond donors should be less than 5 and the numbers of hydrogen bond acceptors should be less than 10. The number of hydrogen bond donors and acceptors were found to be 1 or 2 and 5–6.25, respectively. According to these calculations, all compounds complied with hydrogen bond donor and acceptor groups criteria of RO5. The calculated values for the coefficient of distribution (QPlogP) of the R_1-fluorine and R_2-methyl or ethyl-substituted compounds (**70–79, 81**, **70–79**, **81**, and **82**, except **80**), and R_1-trifluoromethoxy, R_2-hydrogen or methyl, R_3-halogen, methyl or methoxy-substituted compounds (**92**, **93**, **98**, **99**, **102**, and **106**) are in the range of 3.839–4.924 and meet the partition coefficient criteria of RO5. When all these values were examined, it was seen that compounds **70–79**, **81**, **82**, **92**, **93**, **98**, **99**, **102**, and **106** were suitable for all parameters of RO5 and the noncompliance RO5 numbers of the compounds were zero. Both the most potent anti-IL1R inhibitor compounds **76**, **78**, and **81**, as well as the lead compounds **52** and **65** took part in this group. Whereas, in the R_1-fluorine-substituted derivatives, the QPlogP values are above 5 for all the R_2-benzyl-substituted compounds (**83–91**) and the R_2-ethyl-substituted compound (**80**) carrying the trifluoromethoxy group at the thiosemicarbazone moiety. The calculated QPlogP values of the compounds were in the range of 5.066–6.365. In the R_1 trifluoromethoxy substituted derivatives, the QPlogP values of all the R_2 benzyl substituted compounds (**113–117**) and most of the R_2-ethyl-substituted compounds (**105**, **107–111**) are above 5. In addition, the QPlogP values of the compounds (**94–97**, **100**, and **101**) bearing trifluoromethyl, ethyl, t-butyl, thiomethyl, and trifluoromethoxy at the R_3 position, were calculated above 5 in the R_1-trifluoromethoxy and R_2-methyl-substituted derivatives. The non-compliance RO5 numbers of the compounds are one. Therefore, all described compounds have sufficient properties to drug-likeness, and there was no problem in agreement with RO5 for all the compounds as a maximum of one rule is allowed not to comply with at this system.

The predicted values of solubility in water (QPlogS), blood–brain barrier partition coefficient (QPlogBB), central nervous system (CNS) activity, skin permeability (QPlogKp), ratio of binding serum albumin in human (QPlogKhsa) and oral absorption percentage in humans were also examined. The QPlogS values should be in the range of (−6.5) to 0.5. The QPlogKhsa values were determined in the range of (−5.513) to (−6.477) for the compounds **52**, **71**, **72**, **74**, **75**, **77–79**, **81**, **82**, **98**, and **99**, as well as the lead compound **52**, and complied with this criterion in terms of water solubility. Since the other compounds and the lead compound **65** have high lipophilicity, QPlogS values were found to be (−6.575) to (−8.951). The CNS activity of the compound whose QPlogBB value is close to positive values increases, so it can easily cross the blood–brain barrier. The QPlogBB value and CNS activity should be in the ranges of (−3) to 1.2 and (−2) to 2, respectively. The QPlogBB values and CNS activities of the compounds **70–116**, as well as the lead compounds **52** and **65** are on average within the reference ranges from −0.332 to 0.172 and 0 or 1, respectively. These values indicate that most of the compounds have low CNS activities. The QPlogKp and QPlogKhsa values should be in the ranges of −8 to −1 and −1.5 to 1.5, respectively. The values of the compounds **52**, **65**, and **70–116** were suitable for reference with the ranges of (−2.358) to (−0.530) and 0.273–0.951, respectively. The oral absorption percentage

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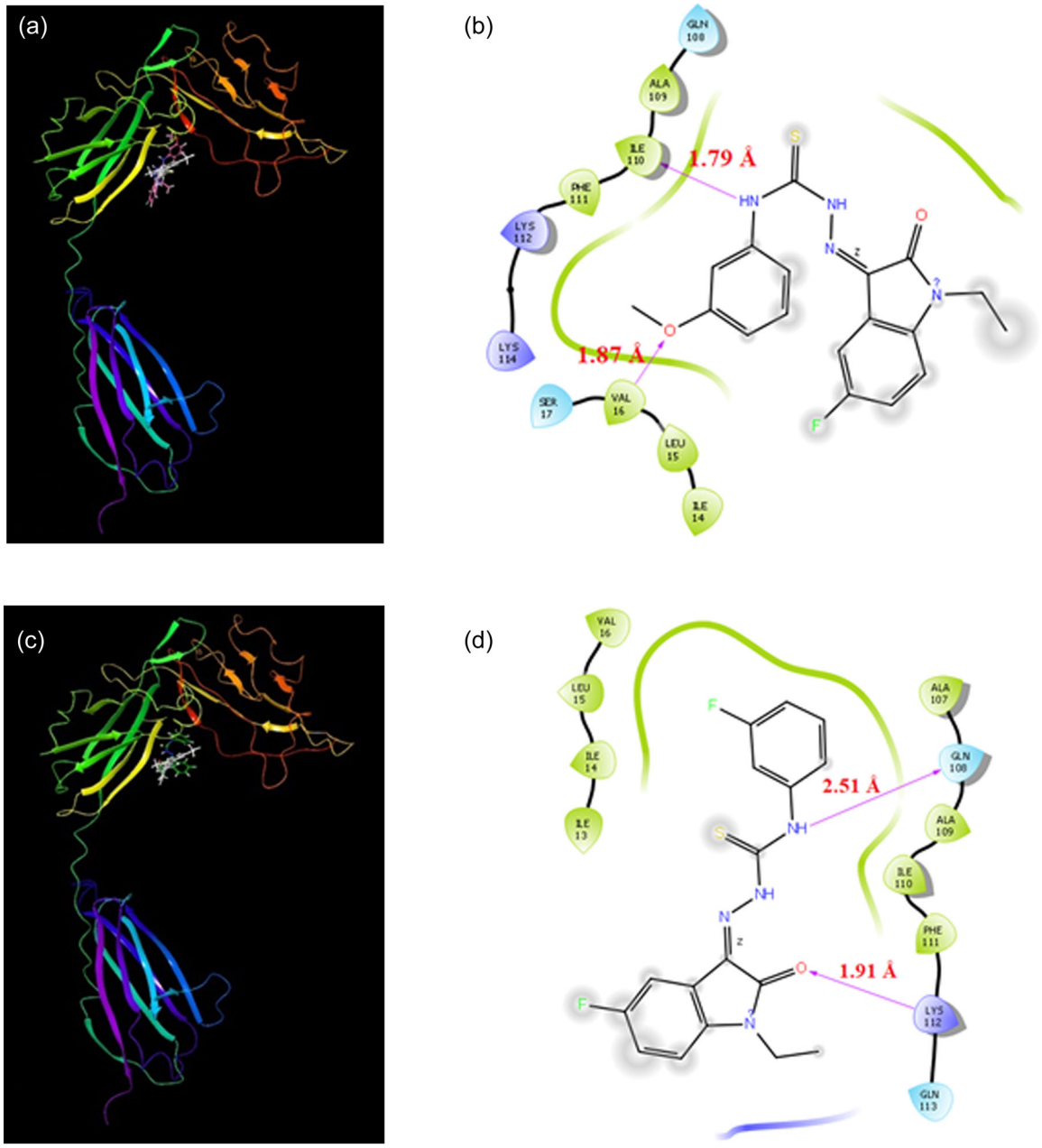


FIGURE 5 Binding modes and interactions of compounds **78** (a, b) and **81** (c, d) with interleukin (IL)-1R1 (PDB ID: 1ITB). In the parts of a and c, compound **65** was seen in white, compound **78** in pink, and compound **81** in green to compare. In the parts of b and d, the distances of the interactions were written near the interactions on the figures.

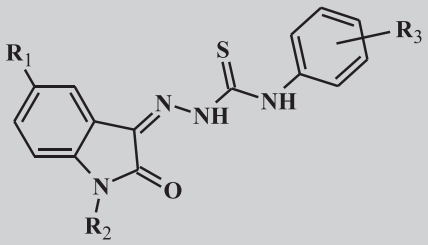
in human has been defined as >80 high and <25 weak and the values were calculated 100% for the compounds **52**, **65**, and **70–116**. These findings show that compounds **71**, **72**, **74**, **75**, **77–79**, **81**, **82**, **98**, and **99** with an RO5 value of 0 are the compounds with the best parameters in terms of drug candidate properties.

2.2 | Chemistry

5-Fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione (compounds **1** and **2**) were reacted with methyl, ethyl, or benzyl halide to get 1-substituted 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-diones (compounds **3–8**). 4-(Substituted phenyl)thiosemicarbazides (compounds **28–46**) have been synthesized by the reaction of substituted phenyl isothiocyanates (compounds **9–27**) with hydrazine hydrate. 1-(Non)substituted 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **47–116**) were obtained by condensation of the compounds **1–8** with the compounds **28–46**.[30,39,42–45] The compounds **47–82** and **99** have been previously described by Güzel et al.,[30] Karalı et al.,[34,46] Sevinçli et al.,[39,42] Soylu-Eter et al.,[43] Ahmad et al.,[44] and Pervez et al.[45] in literature (Experimental information for these molecules was given in the Supplementary Material). Structures of the synthesized new

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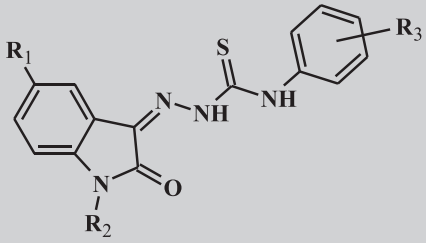
TABLE 2 Lipinski's rule of five and predicted pharmacokinetic properties data of 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-substituted phenylthiosemicarbazones) **52**, **65**, and **70–116**.



Compound	R_1	R_2	R_3	Molecular weight Ref. <500	Number of hydrogen bond donors <5	Number of hydrogen bond acceptors <10	QPlogP octanol/water <5	Number of RO5 rules not complied with ≤1	QPlogS −6.5 to 0.5	QPlogBB −3 to 1.2	CNS −2 inactive; +2 active	QPlogKp −8 to −1	QPlogKhsa −1.5 to 1.5	Oral absorption percentage in human >80 high; <25 weak
52	F	CH_3	3-Cl	362.80	1	5.5	4.047	0	−5.944	−0.031	0	−1.731	0.285	100
65	OCF_3	CH_3	4-CH_3	408.39	1	5.5	4.756	0	−6.874	−0.122	0	−1.749	0.551	100
70	F	CH_3	4-CF_3	396.36	1	5.5	4.541	0	−6.588	0.089	1	−1.78	0.423	100
71	F	CH_3	4-C_2H_5	356.41	1	5.5	4.209	0	−5.853	−0.286	0	−1.708	0.431	100
72	F	CH_3	4-SCH_3	374.45	1	6	4.195	0	−5.905	−0.166	0	−1.688	0.342	100
73	F	CH_3	4-OCF_3	412.36	1	5.5	4.668	0	−6.575	0.033	1	−1.684	0.428	100
74	F	C_2H_5	H	342.39	1	5.5	3.965	0	−5.513	−0.197	0	−1.369	0.273	100
75	F	C_2H_5	4-CH_3	356.41	1	5.5	4.251	0	−5.826	−0.211	0	−1.554	0.428	100
76	F	C_2H_5	4-CF_3	410.38	1	5.5	4.924	0	−6.906	0.083	1	−1.588	0.526	100
77	F	C_2H_5	4-C_2H_5	370.44	1	5.5	4.605	0	−6.477	−0.289	0	−1.497	0.541	100
78	F	C_2H_5	3-OCH_3	372.41	1	6.25	3.839	0	−5.531	−0.281	0	−1.463	0.273	100
79	F	C_2H_5	4-SCH_3	388.47	1	6	4.588	0	−6.465	−0.167	0	−1.473	0.449	100
80	F	C_2H_5	4-OCF_3	426.38	1	5.5	5.066	1	−6.935	0.021	1	−1.489	0.538	100
81	F	C_2H_5	3-F	360.38	1	5.5	4.186	0	−5.702	−0.09	0	−1.506	0.314	100
82	F	C_2H_5	3-Cl	376.83	1	5.5	4.425	0	−6.052	−0.039	0	−1.541	0.387	100
83	F	$C_6H_5CH_2$	3-CH_3	418.48	1	5.5	5.535	1	−7.356	−0.253	0	−0.762	0.833	100
84	F	$C_6H_5CH_2$	4-CH_3	418.48	1	5.5	5.556	1	−7.399	−0.248	0	−0.747	0.838	100

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TABLE 2 (Continued)

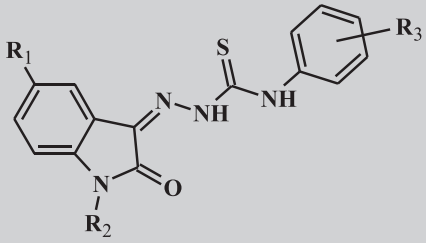


Compound	R_1	R_2	R_3	Molecular weight Ref. <500	Number of hydrogen bond donors <5	Number of hydrogen bond acceptors <10	QPlogP octanol/water <5	Number of RO5 rules not complied with ≤1	QPlogS −6.5 to 0.5	QPlogBB −3 to 1.2	CNS −2 inactive; +2 active	QPlogKp −8 to −1	QPlogKhsa −1.5 to 1.5	Oral absorption percentage in human >80 high; <25 weak
85	F	$C_6H_5CH_2$	4-CF_3	472.45	1	5.5	6.211	1	−8.161	0.06	1	−0.769	0.928	100
86	F	$C_6H_5CH_2$	4-C_2H_5	432.51	1	5.5	5.882	1	−7.676	−0.299	0	−0.686	0.939	100
87	F	$C_6H_5CH_2$	4-OCF_3	488.45	1	5.5	6.365	1	−8.24	−0.014	0	−0.684	0.946	100
88	F	$C_6H_5CH_2$	3-F	422.45	1	5.5	5.466	1	−7.151	−0.109	0	−0.672	0.714	100
89	F	$C_6H_5CH_2$	3-Cl	438.90	1	5.5	5.727	1	−7.535	−0.058	0	−0.708	0.789	100
90	F	$C_6H_5CH_2$	4-Cl	438.90	1	5.5	5.727	1	−7.535	−0.055	0	−0.702	0.788	100
91	F	$C_6H_5CH_2$	4-Br	483.35	1	5.5	5.805	1	−7.654	−0.046	0	−0.707	0.812	100
92	OCF_3	H	3-Cl	414.78	2	5	4.372	0	−6.667	−0.332	0	−2.358	0.403	100
93	OCF_3	CH_3	3-CH_3	408.39	1	5.5	4.754	0	−6.702	−0.123	0	−1.750	0.551	100
94	OCF_3	CH_3	3-CF_3	462.36	1	5.5	5.427	1	−7.712	0.143	1	−1.771	0.656	100
95	OCF_3	CH_3	4-CF_3	462.36	1	5.5	5.448	1	−7.723	0.172	1	−1.768	0.654	100
96	OCF_3	CH_3	4-C_2H_5	422.42	1	5.5	5.129	1	−7.296	−0.202	0	−1.679	0.669	100
97	OCF_3	CH_3	4-$C(CH_3)_3$	450.47	1	5.5	5.698	1	−7.988	−0.209	0	−1.765	0.926	100
98	OCF_3	CH_3	3-OCH_3	424.39	1	6.25	4.533	0	−6.469	−0.185	0	−1.629	0.399	100
99	OCF_3	CH_3	4-OCH_3	424.39	1	6.25	4.542	0	−6.474	−0.159	0	−1.642	0.396	100
100	OCF_3	CH_3	4-SCH_3	440.45	1	6	5.097	1	−7.252	−0.079	0	−1.668	0.572	100
101	OCF_3	CH_3	4-OCF_3	478.36	1	5.5	5.581	1	−7.719	0.120	1	−1.665	0.661	100
102	OCF_3	CH_3	3-F	412.36	1	5.5	4.680	0	−6.661	0.008	1	−1.687	0.435	100

(Continues)

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TABLE 2 (Continued)



Compound	R_1	R_2	R_3	Molecular weight Ref. <500	Number of hydrogen bond donors <5	Number of hydrogen bond acceptors <10	QPlogP octanol/water <5	Number of RO5 rules not complied with ≤1	QPlogS −6.5 to 0.5	QPlogBB −3 to 1.2	CNS −2 inactive; +2 active	QPlogKp −8 to −1	QPlogKhsa −1.5 to 1.5	Oral absorption percentage in human >80 high; <25 weak
103	OCF_3	C_2H_5	3-CH_3	422.42	1	5.5	5.137	1	−7.184	−0.129	0	−1.561	0.656	100
104	OCF_3	C_2H_5	4-CH_3	422.42	1	5.5	5.142	1	−7.191	−0.119	0	−1.546	0.654	100
105	OCF_3	C_2H_5	3-CF_3	476.39	1	5.5	5.818	1	−8.057	0.141	1	−1.582	0.762	100
106	OCF_3	C_2H_5	3-OCH_3	438.42	1	6.25	4.896	0	−6.732	−0.183	0	−1.442	0.495	100
107	OCF_3	C_2H_5	4-SCH_3	454.48	1	6	5.495	1	−7.613	−0.084	0	−1.468	0.681	100
108	OCF_3	C_2H_5	3-F	426.38	1	5.5	5.063	1	−6.984	0.001	1	−1.498	0.539	100
109	OCF_3	C_2H_5	4-F	426.38	1	5.5	5.064	1	−6.923	0.028	1	−1.478	0.535	100
110	OCF_3	C_2H_5	3-Cl	442.84	1	5.5	5.321	1	−7.360	0.052	1	−1.533	0.613	100
111	OCF_3	C_2H_5	4-Cl	442.84	1	5.5	5.338	1	−7.400	0.051	1	−1.519	0.618	100
112	OCF_3	$C_6H_5CH_2$	H	470.46	1	5.5	6.129	1	−7.893	−0.123	0	−0.530	0.901	100
113	OCF_3	$C_6H_5CH_2$	3-CH_3	484.49	1	5.5	6.461	1	−8.486	−0.146	0	−0.724	1.071	100
114	OCF_3	$C_6H_5CH_2$	4-CH_3	484.49	1	5.5	6.461	1	−8.537	−0.156	0	−0.733	1.071	100
115	OCF_3	$C_6H_5CH_2$	4-C_2H_5	498.52	1	5.5	6.828	1	−8.951	−0.243	0	−0.690	1.194	100
116	OCF_3	$C_6H_5CH_2$	3-F	488.45	1	5.5	6.373	1	−8.313	−0.035	0	−0.687	0.951	100

Note: QPlogP: Partition coefficient QPlogS: Solubility in water (S: concentration in saturated solution in water [mol/dm^3]).

QPlogBB: Blood–brain barrier partition coefficient, CNS: central nervous system activity.

QPlogKp: Skin permeability QPlogKhsa: Human serum albumin binding ratio.

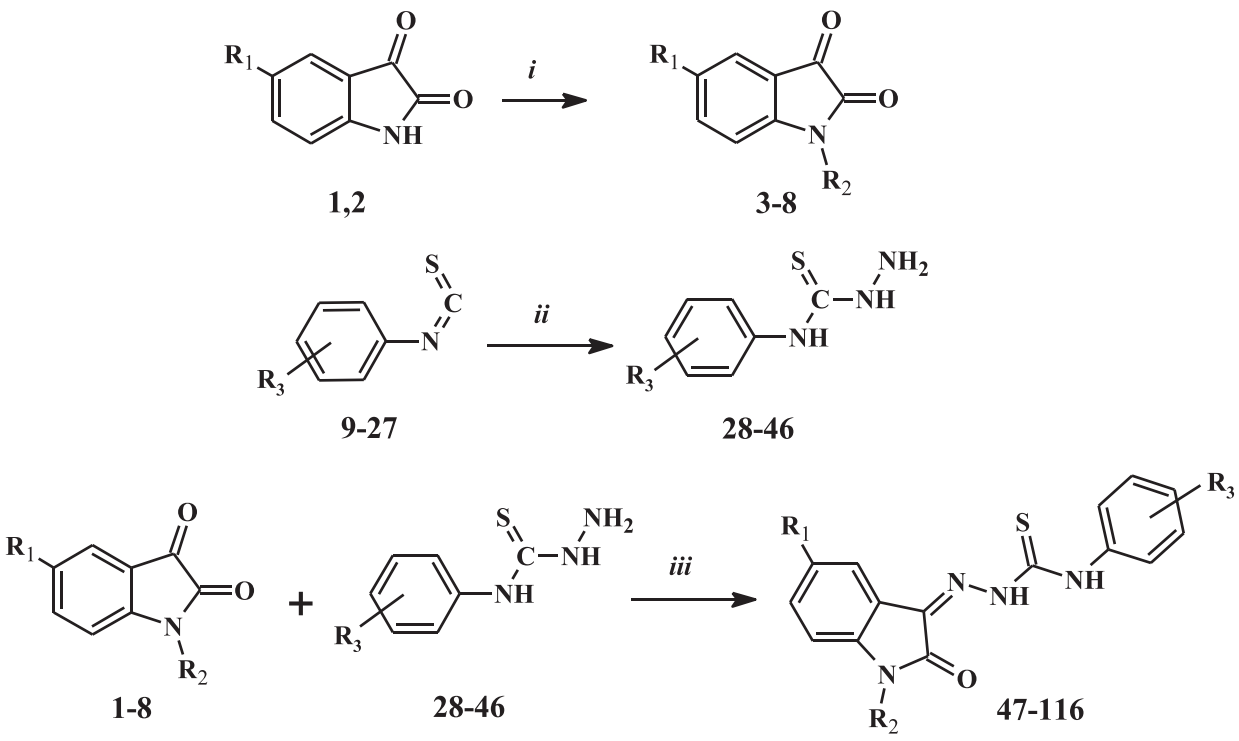
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compounds **83–116** were confirmed by spectral (IR, ^{1}H NMR, ^{13}C NMR, HSQC-2D, HMBC-2D, and MS) and analytical data (Scheme 1, Section 4, and Supporting Information). The isomeric structures of compounds **72** and **99** were determined by X-ray single-crystal diffraction analysis, and the stable isomers were found to be in Z configuration.[42,43]

The new compounds' solid phase (KBr) IR spectra showed in 3134–3361 cm^{-1} region resulting from the NH stretching bands of the thioamide groups. Furthermore, C=S stretching bands observing 1138–1273 cm^{-1} region proved the presence of thioamide groups.

^{1}H NMR spectra displayed the thioamide N_2 and N_4 protons of the thiosemicarbazone moiety as two different singlets at δ 12.55–12.72 ppm and 10.80–11.06 regions, respectively. While the indole proton signals of the compounds **83–91** bearing the fluorine group at the position 5 of the indole ring were observed at low ppm values due to the strong electron donor effect of fluorine, the indole proton signals of the compounds **92–116** bearing the trifluoromethoxy group at the position 5 of the indole ring were observed in the high ppm region. Resonance values of aromatic protons were determined by considering ^{1}H-^{13}C relationships and substituent effects in HSQC-2D and HMBC-2D spectra. In the spectra of the compounds **83–91**, indole C_4-H signals were observed at δ 7.66–7.71 ppm region as a doublet of a doublet. The indole C_6-H signaled at δ 7.20–7.23 ppm as a triplet of doublet, while the indole C_7-H signaled at δ 7.03–7.04 ppm as a doublet of a doublet. In the spectra of compounds bearing the trifluoromethoxy group at position 5 of the indole ring, indole C_4-H signals were observed at δ 7.77–7.88 ppm as a doublet except the compound **104** as singlet. The indole C_6-H was observed as a multiplet at δ 7.36–7.50 ppm alone or at δ 7.36–7.43 ppm with some phenyl protons or as a doublet of doublet at δ 7.34–7.62 ppm or signaled at δ 7.45–7.48 ppm as a doublet of doublet of doublet, too. The indole C_7-H signaled at δ 7.01–7.33 ppm as a doublet. The presence of singlet and single peaks of thioamide N_2 and N_4 proton signals, as well as a single signal of the phenyl protons, and singlet and single peaks of the methyl, ethyl, methoxy, and thiomethyl groups in the phenyl ring confirmed that the compounds were eluded only in Z-isomer form.

All new compounds were subjected to ^{13}C NMR (decoupled) taken in DMSO-d_6 medium analysis to verify the ^{1}H NMR findings of the synthesized compounds. The indole ring and thiosemicarbazide residue carbon signals were determined in the spectra of all compounds, and the confirmation by the interaction constants of the interruption of the indole and phenyl signals by ^{13}C-^{19}F



R_1= F, OCF_3

R_2= H, CH_3, C_2H_5, $CH_2C_6H_5$ (X= Cl, Br, I)

R_3= H, 2-CH_3, 3-CH_3, 4-CH_3, 3-CF_3, 4-CF_3, 4-C_2H_5, 4-$C(CH_3)_3$, 3-OCH_3, 4-OCH_3, 4-SCH_3, 4-OCF_3, 2-F, 3-F, 4-F, 3-Cl, 4-Cl, 4-Br, 4-NO_2

SCHEME 1 Synthesis of 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **47–116**). Reagents and conditions: *i*. (1) R_2X, K_2CO_3, DMF, rt, (2) KI, heat, reflux; *ii*. $NH_2NH_2.H_2O$, C_2H_5OH; *iii*. C_2H_5OH, H_2SO_4.

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interaction supported this data. Further elucidation was done by HSQC-2D and HMBC-2D of prototype selected compounds to identify the carbon signals, and to support ^{1}H NMR, ^{13}C NMR (Decoupled), and DEPT findings. The indole C_2 and C_3 carbons signals showed at δ 160.47–162.80 and 129.94–131.62 ppm regions, respectively. In addition, the C=S signals which verified the thiosemicarbazone structure were seen at the δ 176.11–177.01 ppm region. The HSQC-2D experiments confirmed the resonance of carbons bearing hydrogen in the indole and phenyl rings, while the HMBC-2D analysis confirmed the resonances of the quaternary carbons. Indole C_2 and lactam NH-linked C_{7a} (δ 139.30–143.16 ppm) carbons showed the most downfield shift among the indole carbon signals. The indole C_7 (δ 111.01–112.17 ppm) and C_{3a} (δ 120.55–121.52 ppm) carbons at the *o*-position of the lactam group were found to be the most shielded among the indole carbons. The indole C_5 signals were observed at δ 159.14–159.20 ppm as a doublet in the compounds R_1 fluorine substituted and δ 143.48–144.48 ppm as a quartet in the compounds R_1 trifluoromethoxy substituted due to ^{13}C-^{19}F connections. The OCF_3 signals on the indole ring in the compounds were monitored at δ 120.09–120.68 ppm as a quartet.

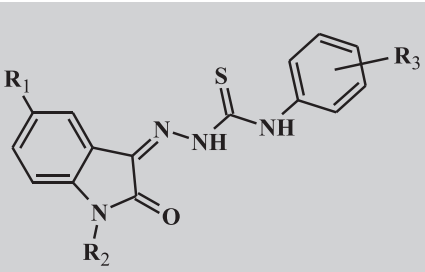
The mass spectra of new compounds were obtained using the positive electrospray ionization (ESI+) technique, $[M+H]^+$ peaks were observed as the base peak in all compounds, and confirmed the molecular weights of the compounds. In addition, $[M+H]^+$+2 peaks were also detected in the spectra of R_3 chlorine or bromine substituted compounds.

2.3 | In vitro IL-1 receptor inhibitory effects

We tested the in vitro IL-1R inhibitory effects of the both previously synthesized 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **47–69**), and newly designed and synthesized 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **70–116**) which include, binding interactions to IL-1R and some drug-like physicochemical properties in silico.

We tested the in vitro activity of the compounds **47–116** on IL-1-mediated activation in HEK cell lines that stably express IL-1R and a NF-κB-secreted alkaline phosphatase (SEAP) reporter. Cells were treated with a fixed concentration of IL-1β (1.3 ng/mL) and increasing concentrations (0.01–20 μM) of the compounds **47–116**. To avoid those compounds that induce nonspecific toxicity, we also assayed the impact on cellular proliferation using an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium bromide)] tetrazolium proliferation assay at a single high concentration of compound (up to 30 μM, at least 50 folds more than the IC_{50}). We showed that the tested compounds exhibit nontoxic IL-1R inhibitor activity at low μM levels. No cytotoxic effect was evident in cell viability experiments. Furthermore, the IL-1-specific SEAP measurements confirmed a potent anti-IL-1 activity of some compounds without affecting the viability of the cells (Tables 3 and 4).

TABLE 3 Anti-interleukin (IL)-1R effects of 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **47–69**).



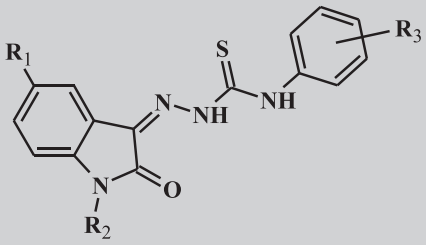
Compound[a]	R_1	R_2	R_3	IL-1R1 (μM)	MTS (μM)
47	F	H	H	>20	>30
48	F	H	4-CH_3	1	>30
49	F	H	4-F	2	>30
50	F	H	4-Cl	1	>30
51	F	CH_3	4-CH_3	0.1	>30
52	F	CH_3	3-Cl	**0.09**	>30
53	OCF_3	H	H	1.1	>30
54	OCF_3	H	2-CH_3	11.8	>30
55	OCF_3	H	3-CH_3	14.5	>30
56	OCF_3	H	4-CF_3	18.7	>30
57	OCF_3	H	4-C_2H_5	13.5	>30
58	OCF_3	H	4-OCH_3	1.2	>30
59	OCF_3	H	2-F	1.1	>30
60	OCF_3	H	3-F	9.8	>30
61	OCF_3	H	4-F	2	>30
62	OCF_3	H	4-Cl	1.3	>30
63	OCF_3	H	4-NO_2	1.6	>30
64	OCF_3	CH_3	H	0.4	>30
65	OCF_3	CH_3	4-CH_3	**0.07**	>30
66	OCF_3	CH_3	4-F	0.1	>30
67	OCF_3	CH_3	4-Cl	0.3	>30
68	OCF_3	CH_3	4-Br	0.13	>30
69	OCF_3	CH_3	4-NO_2	0.2	>30

Note: Bold values indicate the leading compounds.
[a]Defined in the literature.[30,34,39,44,45]

The pre-screening of the compounds **47–69** for the IL-1β inhibitory responses delivered IC_{50} values of 0.07−>20 μM. Generally, no significant difference was observed between fluorine and trifluoromethoxy substituted compounds at position 5 of the indole ring (R_1), while methyl substituted compounds at position 1 of the indole ring (R_2) had higher inhibitory activities than nonsubstituted compounds. The in vitro test results and docking poses were strongly

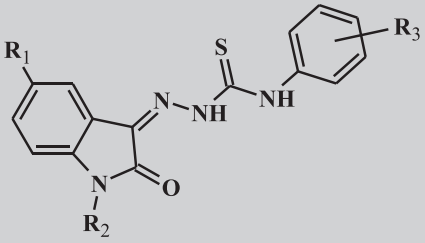
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TABLE 4 Anti-interleukin (IL)-1R effects of 5-fluoro/(trifluoromethoxy)-1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (compounds **70–116**).



Compounds	R_1	R_2	R_3	IL-1R1 (μM)	MTS (μM)
70[a]	F	CH_3	4-CF_3	0.1	>30
71[a]	F	CH_3	4-C_2H_5	3.1	>30
72[a]	F	CH_3	4-SCH_3	0.7	>30
73[a]	F	CH_3	4-OCF_3	0.1	>30
74[a]	F	C_2H_5	H	0.4	>30
75[a]	F	C_2H_5	4-CH_3	1.2	>30
76[a]	F	C_2H_5	4-CF_3	**0.03**	>30
77[a]	F	C_2H_5	4-C_2H_5	1.5	>30
78[a]	F	C_2H_5	3-OCH_3	**0.01**	>30
79[a]	F	C_2H_5	4-SCH_3	0.7	>30
80[a]	F	C_2H_5	4-OCF_3	9.4	>30
81[a]	F	C_2H_5	3-F	**0.02**	>30
82[a]	F	C_2H_5	3-Cl	0.2	>30
83	F	$C_6H_5CH_2$	3-CH_3	13	>30
84	F	$C_6H_5CH_2$	4-CH_3	0.9	>30
85	F	$C_6H_5CH_2$	4-CF_3	3.6	>30
86	F	$C_6H_5CH_2$	4-C_2H_5	3.4	>30
87	F	$C_6H_5CH_2$	4-OCF_3	1.1	>30
88	F	$C_6H_5CH_2$	3-F	3	>30
89	F	$C_6H_5CH_2$	3-Cl	3.2	>30
90	F	$C_6H_5CH_2$	4-Cl	0.8	>30
91	F	$C_6H_5CH_2$	4-Br	**0.06**	>30
92	OCF_3	H	3-Cl	3.9	>30
93	OCF_3	CH_3	3-CH_3	0.2	>30
94	OCF_3	CH_3	3-CF_3	0.1	>30
95	OCF_3	CH_3	4-CF_3	0.1	>30
96	OCF_3	CH_3	4-C_2H_5	0.3	>30
97	OCF_3	CH_3	4-$C(CH_3)_3$	0.1	>30
98	OCF_3	CH_3	3-OCH_3	3.6	>30
99*	OCF_3	CH_3	4-OCH_3	0.3	>30

TABLE 4 (Continued)



Compounds	R_1	R_2	R_3	IL-1R1 (μM)	MTS (μM)
100	OCF_3	CH_3	4-SCH_3	**0.02**	>30
101	OCF_3	CH_3	4-OCF_3	0.4	>30
102	OCF_3	CH_3	3-F	0.1	>30
103	OCF_3	C_2H_5	3-CH_3	1.7	>30
104	OCF_3	C_2H_5	4-CH_3	0.41	>30
105	OCF_3	C_2H_5	3-CF_3	**0.06**	>30
106	OCF_3	C_2H_5	3-OCH_3	2.5	>30
107	OCF_3	C_2H_5	4-SCH_3	**0.02**	>30
108	OCF_3	C_2H_5	3-F	0.7	>30
109	OCF_3	C_2H_5	4-F	0.2	>30
110	OCF_3	C_2H_5	3-Cl	0.7	>30
111	OCF_3	C_2H_5	4-Cl	0.3	>30
112	OCF_3	$C_6H_5CH_2$	H	1.5	>30
113	OCF_3	$C_6H_5CH_2$	3-CH_3	5.77	>30
114	OCF_3	$C_6H_5CH_2$	4-CH_3	2.9	>30
115	OCF_3	$C_6H_5CH_2$	4-C_2H_5	1.4	>30
116	OCF_3	$C_6H_5CH_2$	3-F	3.4	>30

Note: Bold values indicate the prominent values.

[a]Defined in the literature.[39,42,43]

correlated for nonsubstituted derivatives at the R_2 position, which displayed significantly reduced potency (1–>20 μM). Among the fluorine or trifluoromethoxy substituted compounds at the R_1 position, all the 1-methyl-substituted compounds **51**, **52**, and **64–69** at the R_2 position showed inhibitory effects below 1 μM. The IC_{50} values of the compounds **51**, **52**, and **64–69** are 0.1, 0.09, 0.4, 0.07, 0.1, 0.3, 0.13, and 0.2 μM, respectively. The substitutions at positions 3 or 4 of the phenyl ring at the thiosemicarbazone moiety (R_3) significantly increased the inhibitory effect. In 5-fluorine substituted compounds **47–50**, the inhibitory effects were increased by 4-methyl, 4-fluorine and 4-chlorine substitution at R_3. In 5-trifluomethoxy-1-methyl substituted compounds, the inhibitory effect of the non-substituted compound **64** at R_3 was reduced compared to 4-methyl, 4-fluorine, 4-chlorine, 4-bromine, and 4-nitro-substituted compounds **65–69** at R_3, respectively. The 5-fluorine

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substitued-compound **52** bearing a chlorine group at position 3 of phenyl ring and 5-(trifluoromethyl) substituted-compund **65** carrying a methyl group at position 4 of the phenyl ring had an IC_{50} value at nanomolar range, an expected blocker for IL-1 receptors in "in vitro conditions." Based on the test results, the compounds **52** (IC_{50} = 0.09 μM) and **65** (IC_{50} = 0.07 μM) with the strongest inhibitory activity and suitable drug properties were selected as the lead compound. However, the effects were significantly reduced in all 1-nonsubstituted compounds **47–50** and **53–63** (Table 3).

The novel compounds **70–116** were designed and synthesized based on preliminary in vitro test results of the compounds **47–69**, binding interactions to IL-1R and some drug-like physicochemical properties in silico. All compounds tested displayed nontoxic inhibitory effects at IC_{50} values in the range of 10 nM to 13 μM. In vitro test results of compounds **70–116** generally showed that methyl or ethyl substituted compounds at position 1 of the indole ring (R_2) were more effective than nonsubstituted and benzyl substituted compounds. Most compounds bearing methyl or ethyl groups at position 1 of the indole ring (R_2) demonstrated IC_{50} values in the nanomolar range. 1-Benzyl substituted compounds had higher inhibitory effects than nonsubstituted compounds, but mostly lower inhibitory effects than methyl and ethyl substituted compounds. In addition, these compounds (except **91**) were found to have significantly lower potency compared to the lead compounds **52** and **65**. Compounds **76**, **78**, **81**, **91**, **100**, **105**, and **107** showed inhibitory effects on IL-1R-dependent responses in the range of 0.01–0.06 μM and stronger than the lead compounds **52** and **65**. Generally, these compounds are 1-ethylsubstituted compounds (except 1-benzyl substituted **91** and 1-methyl substituted **100**).

Test results showed that compounds **78** (IC_{50} = 0.01 μM), **81** (IC_{50} = 0.02 μM), **100** (IC_{50} = 0.02 μM), and **107** (IC_{50} = 0.02 μM) had the strongest IL-1R inhbitory effects. Among these compounds, only compound **100** carries a methyl group at the R_2 position, whereas all other compounds **78**, **81**, and **107** are ethyl substituted derivatives at the R_2 position. The results show that 3-methoxy, 3-fluorine, 4-thiomethyl substitutions at the R_3 position in the compounds are important for strong inhibitory activity. It is also seen that the trifluoromethyl group at the R_3 position also contributes significantly to the efficiency. The IC_{50} values of compound **76** bearing fluorine at R_1 and 4-trifluoromethyl at R_3, and R_3–3-trifluoromethyl substituted compound **105** bearing trifluoromethoxy at R_1 and 3-trifluoromethyl at R_3 are 0.03 and 0.06 μM, respectively. Compounds with benzyl substituent at R_2 showed high IC_{50} values, and docking experiments did not support the benzyl group at R_2 as best fit the receptor except the compound **91** carrying fluorine at the R_1 and 4-bromine at the R_3. The IC_{50} value of this compound (0.06 μM) is one of the lowest among 1-benzyl-5-fluorine substituted compounds. Another important finding obtained in this study is that, generally, the inhibitory effects are significantly reduced by 3-methyl, 3-methoxy, 4-ethyl, and 4-trifluoromethoxy substitutions at R_3 in R_1-fluorine or trifluoromethoxy-R_2-ethyl substituted compounds (Table 4).

Both COX-1 and COX-2 contribute to IL-1 beta-stimulated prostanoid synthesis.[47] Although our in vitro screening strategy was based on a specific assessment of the activation of IL-1 receptors through a reporter assay without any interference with COX isozymes, it is worthwhile to evaluate whether new molecules interfere with the COX enzyme. Using an inhibitory screening immunoassay kit, we tested the potential COX-1 inhibitory actions of two compounds with the most favorable IC_{50} values, compounds **78** and **81**. The COX-1/2 inhibitor indomethacin was used as a positive control. While the known inhibitor, indomethacin displayed an IC_{50} value of 0.12 pmol/mL, similar to earlier enzymatic studies,[48] both compounds failed to block COX-1, and IC_{50} values cannot be elucidated. Considering the high concentration (30 μM) of the tested compounds, without any success, we decided not to evaluate COX-2 actions at this stage.

3 | CONCLUSION

In this study, we developed a series of new 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) based on the lead compounds **52** and **65** through findings of molecular docking studies and in vitro tests. We evaluated the contribution of substituents at positions 1 and 5 of the indole ring and positions 3 and 4 of the 4-phenylthiosemicarbazone moiety to inhibition of IL-1R. Test results showed no significant difference between fluorine and trifluoromethoxy substituted compounds at position 5 of the indole ring, while methyl or ethyl substituted compounds at position 1 of the indole ring had significantly higher inhibitory efficacy than nonsubstituted and benzyl compounds. In addition, it was determined that most of the compounds containing 3 or 4-trifluoromethyl, 3-methoxy, 4-thiomethyl, 3-fluorine, and 4-bromine groups at the 4-phenylthiosemicarbazone moiety were significant inhibitors of IL-1R. The compounds **76**, **78**, **81**, **91**, **100**, **105**, and **107** had stronger nontoxic inhibitory effects on IL-1R-dependent responses than the lead compounds **52** and **65**. In vitro and in silico findings showed that compounds **78** and **81** had the strongest IL-1R inhbitory effects and the most favorable drug-like properties, and can be selected as target compounds for mechanistic and in vivo studies. Whereas, considering the similarities between TLR2 and IL-1 receptors, and the fact that both IL-1 and TLR ligands produce indistinguishable responses during some important inflammatory events such as the induction of COX or nitric oxide,[49] further studies are needed to discriminate the specificity of the new compounds regarding these two receptor types.

4 | EXPERIMENTAL

4.1 | Molecular modeling

All synthesized compounds were subjected to docking studies on Maestro implemented in the Schrödinger-Small-Molecule Drug Discovery Suite package program (v.2018-3).[50]

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4.1.1 | Protein selection and preparation

The crystallographic structure of IL-1R1 was obtained from Protein Data Bank (PDB database, www.rcsb.org) with the code 1ITB.pdb (2.5 Å) to determine the binding mode interactions for ligands. This crystal structure includes IL-1R1 in complex with the ligand IL-1β.[40] The crystal structure of the complex was cleaned, native ligand IL-1β and the crystallization water molecules, salts, and other small molecules were removed in order not to affect the protein–ligand binding relations, and all hydrogen atoms were added. The structures were preprocessed, filled in missing loops and side chains, optimized of hydrogen-bonded structures, and minimized at pH 7.0 by Protein Preparation Wizard application in Maestro. The IL-1R1 structure was acquired as mae file.

4.1.2 | Ligand preparation

The 2D structures of compounds were drawn by 2D Sketcher application, all hydrogen atoms were added, energy minimization was done at pH 7.0 according to OPLS3e method, and 3D ligand structures were created, and saved as mae file with LigPrep application.

4.1.3 | Molecular docking method

The binding site on IL-1R1 was determined by the model using the most effective lead molecule 1-methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-methylphenyl)-thiosemicarbazone] (compound **65**) as a template according to the results of in vitro tests with novel 2-indolinone derivatives correlated with the free binding energy levels to IL-1R1 in in silico docking experiments. This region was similar to the regions of which IL-1β and IL-1 receptor antagonist anakinra bind, and it presented the importance of Lys112 amino acid in binding. The grid was generated in the center of Lys112 amino acid by Receptor Grid Generation application. The prepared ligands were docking on protein structure with XP scanning method in Glide application. Each ligand was screened 50 times to find high-score binding poses, and five poses showing the highest score for each ligand were recorded for analysis.

4.1.4 | Prediction of physicochemical properties

All compounds were applied to Qikprop application to evaluate compliance with Lipinski's RO5 which is an important standard for the druglikeness and some physicochemical properties relating to absorption, distribution, metabolism, and excretion (ADME).[41]

4.2 | Chemistry

4.2.1 | General

All chemicals, reagents, and solvents were purchased from Sigma-Aldrich (USA) or Merck (Germany). The processes of the reactions were monitored using thin-layer chromatography (TLC). Silica gel 60 HF254 aluminum sheets and solvent system composed of ethylacetate: cyclohexane (50:50, v/v) was used for TLC. UV lamp (Mineralight Lamp UVGL-58) were used at 254 nm for monitoring stains on the TLC plates. The melting points of the synthesized compounds were recorded in open capillaries and uncorrected using a Buchi melting point apparatus B-540. The IR spectra were determined by a Shimadzu IR Affinity-1 FTIR spectrophotometer in KBr tablets. ^{1}H NMR, ^{13}C NMR, HSQC-2D, and HMBC-2D spectra were measured on Varian UNITY INOVA 500 MHz, Varian Mercury (Agilent) 400 MHz, Varian Mercury 300 MHz, and Oxford Pulsar 60 MHz NMR spectrophotometers in DMSO-d_6. The mass spectra were recorded on a Waters 2695 Alliance Micromass ZQ LC/MS, Thermo Scientific TSQ-Quantum Access and Agilent 1260 InfinityLab II LC/MSD spectrophotometers. The elemental analysis was recorded on Leco CHNS-932 and Thermo Finnigan Flash EA 1112 elemental analyzers.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.2.2 | General procedure for the synthesis of 1-substituted 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-diones (**3–8**)

5-Fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione (compounds **1** and **2**) (5 mmol) and anhydrous potassium carbonate (K_2CO_3) (7 mmol, 0.97 g) were continuously stirred in 10 mL of dimethylformamide (DMF) at room temperature for 1 h. After the addition of iodomethane/bromoethane/benzyl chloride (15 mmol) and anhydrous potassium iodide (KI) (1 mmol, 0.17 g) as a catalyst to the mixture, the reaction was heated to 50–60°C under reflux with continuous stirring 1–3 h. The reaction mixture was first evaporated to dryness under reduced pressure to obtain crude product. The obtained solid product was washed with iced water to eliminate excess K_2CO_3 and KI, and purified by crystallization with ethanol.[51–56]

4.2.3 | General procedure for the synthesis of 4-(substituted phenyl)thiosemicarbazides (**28–46**)

A solution of substituted phenyl isothiocyanates (compounds **9–27**) (5 mmol) in 10 mL of ethanol were added dropwise with vigorous stirring to a solution of hydrazine hydrate (5 mmol) in 10 mL of ethanol with iced water cooling. The precipitated solid product was filtered off immediately, and purified by crystallization with ethanol.[57–61]

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4.2.4 | General procedure for the synthesis of 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (**47–116**)

4-(Substituted phenyl) thiosemicarbazide derivatives (compounds **28–46**) (2.5 mmol) were added to a solution of 1-(non)substituted 5-fluoro/(trifluoromethoxy)−1*H*-indole-2,3-diones (compounds **1–8**) (2.5 mmol) in 10 mL of absolute ethanol and trace amounts of concentrated sulphuric acid in ethanol (100 mL) was added occasionally as a catalyst to increase efficiency. The reaction mixture was heated to 80–90°C under reflux until the completion of the reaction. The precipitated crude product was filtered off and washed with or recrystallized from ethanol. The compounds **47–82** and **99** have been previously described by Güzel et al.,[30] Karalı et al.,[34,46] Sevinçli et al.,[39,42] Soylu-Eter et al.,[43] Ahmad et al.,[44] and Pervez et al.[45]

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(3-methylphenyl) thiosemicarbazone] (**83**): Orange powder (93%): m.p.: 181–183.5°C. IR (KBr) ν_{maks} (cm^{-1}): 3253, 3219 (NH), 1681 (C=O), 1647, 1608, 1544, 1475 (C=N, C=C), 1269 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 2.34 (3H, s, CH_3); 4.99 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.1 Hz, ind. C_7-H); 7.10 (1H, d, J: 7.6 Hz, phen. C_4-H); 7.21 (1H, td, J: 9.1; 2.7 Hz, ind. C_6-H); 7.25-7.39 (6H, m, benz. $C_{2,3,4,5,6}$-H, phen. C_5-H); 7.40 (1H, s, phen. C_2-H); 7.43 (1H, d, J: 8.0 Hz, phen. C_6-H); 7.71 (1H, dd, J: 8.1; 2.7 Hz, ind. C_4-H); 10.84 (1H, s, N_4-H); 12.58 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/125 MHz) δ (ppm): 21.40 (CH_3); 43.12 (ind. N-$CH_2C_6H_5$); 108.85 (d, J: 26.3 Hz, ind. C_4); 112.05 (d, J: 8.4 Hz, ind. C_7); 117.79 (d, J: 24.2 Hz, ind. C_6); 121.52 (d, J: 10.3 Hz, ind. C_{3a}); 123.16 (phen. C_6); 126.47 (phen. C_2); 127.40 (phen. C_4); 127.93 (benz. $C_{2,6}$); 128.14 (benz. C_4); 128.78 (phen. C_5); 129.20 (benz. $C_{3,5}$); 130.94 (d, J: 3.2 Hz, ind. C_3); 136.04 (benz. C_1); 138.33 (phen. C_1); 138.62 (phen. C_3); 139.39 (ind. C_{7a}); 159.20 (d, J: 238.5 Hz, ind. C_5); 161.39 (ind. C_2); 176.62 (CS). Anal. Calcd for $C_{23}H_{19}FN_4OS.^1/_4H_2O$: C, 65.30; H, 5.00; N, 13.24; S, 7.58; Found: C, 65.53; H, 4.68; N, 13.41; S, 7.76.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(4-methylphenyl) thiosemicarbazone] (**84**): Yellow powder (92%): m.p.: 227–232°C. IR (KBr) ν_{maks} (cm^{-1}): 3265, 3226 (NH), 1681 (C=O), 1622, 1597, 1541, 1477 (C=N, C=C), 1269 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 2.32 (3H, s, CH_3); 4.98 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.1 Hz, ind. C_7-H); 7.18-7.24 (1H, m, ind. C_6-H); 7.23 (2H, d, J: 8.3 Hz, phen. $C_{3,5}$-H); 7.26-7.29 (1H, m, benz. C_4-H); 7.33 (2H, t, J: 7.3 Hz, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 7.0 Hz, benz. $C_{2,6}$-H); 7.47 (2H, d, J: 8.3 Hz, phen. $C_{2,6}$-H); 7.70 (1H, dd, J: 8.1; 2.7 Hz, ind. C_4-H); 10.84 (1H, s, N_4-H); 12.57 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/125 MHz) δ (ppm): 21.12 (CH_3); 43.13 (ind. N-$CH_2C_6H_5$); 108.83 (d, J: 26.2 Hz, ind. C_4); 112.01 (d, J: 8.2 Hz, ind. C_7); 117.74 (d, J: 24.4 Hz, ind. C_6); 121.52 (d, J: 9.5 Hz, ind. C_{3a}); 125.99 (phen. $C_{2,6}$); 127.93 (benz. $C_{2,6}$); 128.14 (benz. C_4); 129.20 (benz. $C_{3,5}$); 129.41 (phen. $C_{3,5}$); 130.83 (d, J: 3.1 Hz, ind. C_3); 136.04 (benz. C_1); 136.05 (phen. C_1); 136.19 (phen. C_4); 139.33 (ind. C_{7a}); 159.20 (d, J: 238.7 Hz, ind. C_5); 161.37 (ind. C_2); 176.72 (CS). Anal. Calcd for $C_{23}H_{19}FN_4OS$: C, 66.01; H, 4.58; N, 13.39; S, 7.66; Found: C, 66.08; H, 4.50; N, 13.40; S, 7.72.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(4-trifluoromethylphenyl)thiosemicarbazone] (**85**): Orange powder (58%): m.p.: 181–183°C. IR (KBr) ν_{maks} (cm^{-1}): 3277, 3219 (NH), 1697 (C=O), 1620, 1600, 1544, 1479 (C=N, C=C), 1273 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 4.99 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.04 (1H, dd, J: 8.7; 4.0 Hz, ind. C_7-H); 7.23 (1H, td, J: 9.1; 2.7 Hz, ind. C_6-H); 7.26-7.29 (1H, m, benz. C_4-H); 7.33 (2H, t, J: 7.2 Hz, benz. $C_{3,5}$-H); 7.39 (2H, d, J: 6.9 Hz, benz. $C_{2,6}$-H); 7.69 (1H, dd, J: 8.0; 2.7 Hz, ind. C_4-H); 7.80 (2H, d, J: 8.6 Hz, phen. $C_{2,6}$-H); 7.93 (2H, d, J: 8.4 Hz, phen. $C_{3,5}$-H); 11.05 (1H, s, N_4-H); 12.72 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/125 MHz) δ (ppm): 43.16 (ind. N-$CH_2C_6H_5$); 108.94 (d, J: 26.0 Hz, ind. C_4); 112.12 (d, J: 8.1 Hz, ind. C_7); 118.04 (d, J: 24.1 Hz, ind. C_6); 121.33 (d, J: 9.4 Hz, ind. C_{3a}); 124.65 (d, J: 271.81 Hz, CF_3); 125.97 (phen. $C_{2,6}$); 126.05 (q, J: 3.7 Hz, phen. $C_{3,5}$); 126.59 (q, J: 32.1 Hz, phen. C_4); 127.95 (benz. $C_{2,6}$); 128.15 (benz. C_4); 129.20 (benz. $C_{3,5}$); 131.62 (d, J: 3.6 Hz, ind. C_3); 135.97 (benz. C_1); 139.54 (ind. C_{7a}); 142.46 (phen. C_1); 159.20 (d, J: 238.8 Hz, ind. C_5); 161.39 (ind. C_2); 176.75 (CS). Anal. Calcd for $C_{23}H_{16}F_4N_4OS$: C, 58.47; H, 3.41; N, 11.86; S, 6.79; Found: C, 58.13; H, 3.74; N, 11.58; S, 6.34.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(4-ethylphenyl) thiosemicarbazone] (**86**): Yellow powder (96%): m.p.: 203–205°C. IR (KBr) ν_{maks} (cm^{-1}): 3261, 3226 (NH), 1680 (C=O), 1618, 1595, 1539, 1477 (C=N, C=C), 1269 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.19 (3H, t, J: 7.6 Hz, $CH_2\underline{CH_3}$); 2.62 (2H, q, J: 7.6 Hz, $\underline{CH_2}CH_3$); 4.98 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.0 Hz, ind. C_7-H); 7.20 (1H, td, J: 9.2; 2.7 Hz, ind. C_6-H); 7.25-7.29 (1H, m, benz. C_4-H); 7.26 (2H, d, J: 8.2 Hz, phen. $C_{3,5}$-H); 7.33 (2H, t, J: 7.3 Hz, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 6.9 Hz, benz. $C_{2,6}$-H); 7.50 (2H, d, J: 8.3 Hz, phen. $C_{2,6}$-H); 7.70 (1H, dd, J: 8.1; 2.7 Hz, ind. C_4-H); 10.82 (1H, s, N_4-H); 12.58 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/100 MHz) δ (ppm): 16.07 (CH_3); 28.20 (CH_2); 43.08 (ind. N-$CH_2C_6H_5$); 108.78 (d, J: 25.7 Hz, ind. C_4); 112.01 (d, J: 7.8 Hz, ind. C_7); 117.72 (d, J: 23.8 Hz, ind. C_6); 121.48 (d, J: 9.5 Hz, ind. C_{3a}); 126.01 (phen. $C_{2,6}$); 127.89 (benz. $C_{2,6}$); 128.10 (benz. C_4); 128.18 (phen. $C_{3,5}$); 129.17 (benz. $C_{3,5}$); 130.85 (d, J: 3.4 Hz, ind. C_3); 136.01 (benz. C_1); 136.32 (phen. C_1); 139.30 (ind. C_{7a}); 142.34 (phen. C_4); 159.14 (d, J: 238.9 Hz, ind. C_5); 161.34 (ind. C_2); 176.64 (CS). HRMS (ESI (+)) $C_{24}H_{22}FN_4OS$ $[M+H]^+$: 433.14929; Found $[M+H]^+$: 433.15091. Anal. Calcd for $C_{24}H_{21}FN_4OS.^1/_4H_2O$: C, 65.96; H, 5.30; N, 12.82; S, 7.33; Found: C, 65.89; H, 4.92; N, 12.75; S, 7.87.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(4-trifluoromethoxyphenyl)thiosemicarbazone] (**87**): Yellow powder (82%): m.p.: 156–158°C. IR (KBr) ν_{maks} (cm^{-1}): 3280, 3234 (NH), 1697 (C=O), 1606, 1558, 1508, 1473 (C=N, C=C), 1269 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 4.99 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.04 (1H, dd, J: 8.7; 4.0 Hz, ind. C_7-H); 7.22 (1H, td, J: 9.3; 2.7 Hz, ind. C_6-H); 7.25-7.29 (1H, m, benz. C_4-H); 7.33 (2H, t, J: 7.3 Hz, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 7.2 Hz, benz. $C_{2,6}$-H); 7.44 (2H, d, J: 8.3 Hz, phen. $C_{3,5}$-H); 7.67 (1H, dd, J: 8.0; 2.7 Hz, ind. C_4-H); 7.75 (2H, d, J: 9.0 Hz, phen. $C_{2,6}$-H); 10.97 (1H, s, N_4-H); 12.65 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/125 MHz) δ (ppm): 43.14 (ind. N-$CH_2C_6H_5$); 108.82 (d, J: 26.2 Hz, ind. C_4); 112.12 (d, J: 8.2 Hz, ind. C_7); 117.95 (d, J: 24.2 Hz, ind. C_6); 120.56 (d, J: 256.3 Hz, OCF_3); 121.40 (d, J: 9.4 Hz, ind. C_{3a}); 121.65

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(phen. $C_{2,6}$); 127.88 (phen. $C_{3,5}$); 127.94 (benz. $C_{2,6}$); 128.14 (benz. C_4); 129.20 (benz. $C_{3,5}$); 131.38 (d, J: 3.5 Hz, ind. C_3); 136.0 (benz. C_1); 137.91 (phen. C_4); 139.49 (ind. C_{7a}); 146.43 (d, J: 1.7 Hz, phen. C_1); 159.19 (d, J: 238.4 Hz, ind. C_5); 161.38 (ind. C_2); 176.99 (CS). Anal. Calcd for $C_{23}H_{16}F_4N_4O_2S.^1/_4H_2O$: C, 56.03; H, 3.68; N, 11.36; S, 6.50; Found: C, 56.16; H, 3.32; N, 11.30; S, 6.12.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(3-fluorophenyl) thiosemicarbazone] (**88**): Orange powder (73%): m.p.: 205–207°C. IR (KBr) ν_{maks} (cm^{-1}): 3317, 3197 (NH), 1683 (C=O), 1622, 1602, 1558, 1479 (C=N, C=C), 1269 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 4.98 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.0 Hz, ind. C_7-H); 7.10–7.15 (1H, m, phen. C_4-H); 7.22 (1H, td, J: 9.1; 2.8 Hz, ind. C_6-H); 7.25–7.29 (1H, m, benz. C_4-H); 7.33 (2H, t, J: 7.3 Hz, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 7.1 Hz, benz. $C_{2,6}$-H); 7.44–7.52 (2H, m, phen. $C_{5,6}$-H); 7.62 (1H, dt, J: 10.9; 2.1 Hz, phen. C_2-H); 7.70 (1H, dd, J: 8.0; 2.7 Hz, ind. C_4-H); 10.93 (1H, s, N_4-H); 12.66 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/125 MHz) δ (ppm): 43.14 (ind. N-$CH_2C_6H_5$); 108.91 (d, J: 26.0 Hz, ind. C_4); 112.12 (d, J: 8.1 Hz, ind. C_7); 112.75 (d, J: 24.7 Hz, phen. C_2); 113.36 (d, J: 20.9 Hz, phen. C_4); 117.98 (d, J: 24.1 Hz, ind. C_6); 121.38 (d, J: 9.4 Hz, ind. C_{3a}); 121.76 (d, J: 2.3 Hz, phen. C_6); 127.94 (benz. $C_{2,6}$); 128.14 (benz. C_4); 129.20 (benz. $C_{3,5}$); 130.52 (d, J: 9.7 Hz, phen. C_5); 131.41 (d, J: 3.1 Hz, ind. C_3); 136.0 (benzy C_1); 139.51 (ind. C_{7a}); 140.42 (d, J: 11.2 Hz, phen. C_1); 159.19 (d, J: 238.4 Hz, ind. C_5); 161.40 (ind. C_2); 162.12 (d, J: 242.8 Hz, phen. C_3); 176.65 (CS). Anal. Calcd for $C_{22}H_{16}F_2N_4OS$: C, 62.55; H, 3.82; N, 13.26; S, 7.59; Found: C, 62.37; H, 3.83; N, 13.28; S, 7.58.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(3-chlorophenyl) thiosemicarbazone] (**89**): Orange powder (72%): m.p.: 195–196.5°C. IR (KBr) ν_{maks} (cm^{-1}): 3271, 3213 (NH), 1681 (C=O), 1620, 1587, 1537, 1479 (C=N, C=C), 1267 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 4.98 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.0 Hz, ind. C_7-H); 7.22 (1H, td, J: 9.1; 2.7 Hz, ind. C_6-H); 7.25-7.29 (1H, m, benz. C_4-H); 7.31–7.35 (3H, m, phen. C_4-H, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 7.1 Hz, benz. $C_{2,6}$-H); 7.46 (1H, t, J: 8.1 Hz, phen. C_5-H); 7.66 (1H, ddd, J: 8.1; 2.0; 0.9 Hz, phen. C_6-H); 7.70 (1H, dd, J: 8.0; 2.7 Hz ind. C_4-H); 7.79 (1H, t, J: 2.0 Hz, phen. C_2-H); 10.93 (1H, s, N_4-H); 12.66 (1H, s, N_2-H). ^{13}C NMR (Decoupled, DMSO-d_6/100 MHz) δ (ppm): 43.12 (ind. N-$CH_2C_6H_5$); 108.85 (d, J: 25.8 Hz, ind. C_4); 112.10 (d, J: 8.3 Hz, ind. C_7); 117.96 (d, J: 24.5 Hz, ind. C_6); 121.35 (d, J: 9.0 Hz, ind. C_{3a}); 124.44 (phen. C_6); 125.46 (phen. C_2); 126.46 (phen. C_4); 127.90 (benz. $C_{2,6}$); 128.11 (benz. C_4); 129.17 (benz. $C_{3,5}$); 130.53 (phen. C_5); 131.44 (d, J: 3.9 Hz, ind. C_3); 132.99 (phen. C_3); 135.96 (benz. C_1); 139.48 (ind. C_{7a}); 140.17 (phen. C_1); 159.15 (d, J: 237.2 Hz, ind. C_5); 161.37 (ind. C_2); 176.70 (CS). Anal. Calcd for $C_{22}H_{16}ClFN_4OS$: C, 60.20; H, 3.67; N, 12.77; S, 7.31; Found: C, 60.28; H, 3.49; N, 12.94; S, 7.35.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(4-chlorophenyl) thiosemicarbazone] (**90**): Brown powder (60%): m.p.: 205–208°C. IR (KBr) ν_{maks} (cm^{-1}): 3309, 3269, 3228 (NH), 1693 (C=O), 1593, 1541, 1492, 1477 (C=N, C=C), 1269 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 4.98 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.1 Hz, ind. C_7-H); 7.21 (1H, td, J: 9.1; 2.7 Hz, ind. C_6-H); 7.24–7.29 (1H, m, benz. C_4-H); 7.33 (2H, t, J: 7.2 Hz, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 7.1 Hz, benz. $C_{2,6}$-H); 7.49 (2H, d, J: 8.8 Hz, phen. $C_{3,5}$-H); 7.66 (2H, d, J: 8.7 Hz, phen. $C_{2,6}$-H); 7.66–7.69 (1H, m, ind. C_4-H); 10.92 (1H, s, N_4-H); 12.64 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 43.13 (ind. N-$CH_2C_6H_5$); 108.84 (d, J: 26.0 Hz, ind. C_4); 112.12 (d, J: 8.3 Hz, ind. C_7); 117.94 (d, J: 24.2 Hz, ind. C_6); 121.42 (d, J: 9.8 Hz, ind. C_{3a}); 127.78 (phen. $C_{2,6}$); 127.94 (benz. $C_{2,6}$); 128.15 (benz. C_4); 128.91 (phen. $C_{3,5}$); 129.21 (benz. $C_{3,5}$); 130.78 (phen. C_4); 131.34 (d, J: 3.1 Hz, ind. C_3); 136.01 (benz. C_1); 137.73 (phen. C_1); 139.48 (ind. C_{7a}); 159.18 (d, J: 238.8 Hz, ind. C_5); 161.39 (ind. C_2); 176.86 (CS). Anal. Calcd for $C_{22}H_{16}ClFN_4OS$: C, 60.20; H, 3.67; N, 12.77; S, 7.31; Found: C, 60.21; H, 3.71; N, 12.87; S, 7.46.

1-Benzyl-5-fluoro-1*H*-indole-2,3-dione 3-[4-(4-bromophenyl) thiosemicarbazone] (**91**): Orange powder (69%): m.p.: 220–222°C. IR (KBr) ν_{maks} (cm^{-1}): 3267, 3234 (NH), 1687 (C=O), 1587, 1554, 1475 (C=N, C=C), 1267 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 4.98 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.03 (1H, dd, J: 8.7; 4.0 Hz, ind. C_7-H); 7.21 (1H, td, J: 9.1; 2.7 Hz, ind. C_6-H); 7.25–7.29 (1H, m, benz. C_4-H); 7.33 (2H, t, J: 7.2 Hz, benz. $C_{3,5}$-H); 7.38 (2H, d, J: 6.8 Hz, benz. $C_{2,6}$-H); 7.61 (4H, s, phen. $C_{2,3,5,6}$-H); 7.68 (1H, dd, J: 8.0; 2.7 Hz, ind. C_4-H); 10.89 (1H, s, N_4-H); 12.65 (1H, s, N_2-H). ^{13}C NMR (Decoupled, DMSO-d_6/100 MHz) δ (ppm): 43.10 (ind. N-$CH_2C_6H_5$); 108.82 (d, J: 25.9 Hz, ind. C_4); 112.06 (d, J: 8.3 Hz, ind. C_7); 117.89 (d, J: 24.1 Hz, ind. C_6); 119.03 (phen. C_4); 121.37 (d, J: 9.5 Hz, ind. C_{3a}); 127.90 (benz. $C_{2,6}$); 128.00 (phen. $C_{2,6}$); 128.11 (benz. C_4); 129.16 (benz. $C_{3,5}$); 131.27 (d, J: 3.4 Hz, ind. C_3); 131.79 (phen. $C_{3,5}$); 135.96 (benz. C_1); 138.12 (phen. C_1); 139.42 (ind. C_{7a}); 159.14 (d, J: 238.5 Hz, ind. C_5); 161.33 (ind. C_2); 176.69 (CS). HRMS $C_{22}H_{17}BrFN_4OS$ (ESI (+)) $[M+H]^+$: 483.02850, 485.02645; Found $[M+H]^+$: 483.03009, 485.02798. Anal. Calcd for $C_{22}H_{16}BrFN_4OS$: C, 54.67; H, 3.34; N, 11.59; S, 6.63; Found: C, 54.75; H, 3.09; N, 11.94; S, 6.71.

5-(Trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-chlorophenyl) thiosemicarbazone] (**92**): Orange powder (88%): m.p.: 223–224°C. IR (KBr) ν_{maks} (cm^{-1}): 3309, 3250 (NH), 1697 (C=O), 1629, 1591, 1541, 1479 (C=N, C=C), 1139 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 7.02 (1H, d, J: 8.7 Hz, ind. C_7-H); 7.35 (1H, ddd, J: 8.2; 2.3; 1.2 Hz, phen. C_4-H); 7.37 (1H, ddd, J: 8.6; 2.4; 1.2 Hz, ind. C_6-H); 7.46 (1H, dd, J: 8.2; 7.8 Hz, phen. C_5-H); 7.62 (1H, ddd, J: 8.2; 2.0; 0.8 Hz, phen. C_6-H); 7.76 (1H, dd, J: 2.3; 2.0 Hz, phen. C_2-H); 7.77 (1H, d, J: 2.0 Hz, ind. C_4-H); 10.91 (1H, s, N_4-H); 11.42 (1H, s, ind. NH), 12.71 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 112.16 (ind. C_7); 114.50 (ind. C_4); 120.13 (q, J: 255.5 Hz, OCF_3); 121.23 (ind. C_{3a}); 124.21 (phen. C_6); 124.32 (ind. C_6); 125.21 (phen. C_2); 125.99 (phen. C_4); 129.98 (phen. C_5); 131.55 (ind. C_3); 132.45 (phen. C_3); 139.65 (phen. C_1); 141.43 (ind. C_{7a}); 143.48 (q, J: 1.9 Hz, ind. C_5); 162.71 (ind. C_2); 176.24 (CS). LC-MS (ESI (+)) $C_{16}H_{11}ClF_3N_4O_2S$ $[M+H]^+$: 415.796801; Found $[M+H]^+$: 414.90, 416.90. Anal. Calcd for $C_{16}H_{10}ClF_3N_4O_2S$: C, 46.33; H, 2.43; N, 13.51; S, 7.73; Found: C, 45.99; H, 2.40; N, 13.46; S, 7.78.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-methylphenyl)thiosemicarbazone] (**93**): Bright yellow crystals (95%): m.p.: 203–205°C. IR (KBr) ν_{maks} (cm^{-1}): 3311, 3250 (NH), 1689 (C=O), 1622, 1614, 1552, 1482 (C=N, C=C), 1149 (C=S). ^{1}H NMR

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(DMSO-d_6/400 MHz) δ (ppm): 2.33 (3H, s, CH_3); 3.24 (3H, s, ind. N-CH_3); 7.10 (1H, d, J: 7.4 Hz, phen. C_4-H); 7.25 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.31 (1H, t, J: 7.8 Hz, phen. C_5-H); 7.37–7.42 (2H, m, phen. $C_{2,6}$-H); 7.46 (1H, ddd, J: 8.6; 2.4; 0.8 Hz, ind. C_6-H); 7.83 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.85 (1H, s, N_4-H); 12.55 (1H, s, N_2-H). ^{13}C NMR (Decoupled, HMBC-2D, DMSO-d_6/100 MHz) δ (ppm): 20.87 (CH_3); 25.90 (ind. N-CH_3); 111.01 (ind. C_7); 114.21 (ind. C_4); 120.18 (q, J: 255.5 Hz, OCF_3); 120.73 (ind. C_{3a}); 122.88 (phen. C_6); 123.97 (ind. C_6); 126.17 (phen. C_2); 126.95 (phen. C_4); 128.26 (phen. C_5); 130.17 (ind. C_3); 137.83 (phen. C_1); 138.11 (phen. C_3); 142.50 (ind. C_{7a}); 143.98 (q, J: 1.9 Hz, ind. C_5); 160.88 (ind. C_2); 176.21 (CS). MS (ESI (+)) $C_{18}H_{16}F_3N_4O_2S$ $[M+H]^+$: 409.40490; Found $[M+H]^+$: 409.58. Anal. Calcd for $C_{18}H_{15}F_3N_4O_2S$: C, 52.94; H, 3.70; N, 13.72; S, 7.85; Found: C, 52.64; H, 3.77; N, 13.64; S, 7.88.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-trifluoromethylphenyl)-thiosemicarbazone] (**94**): Bright yellow amorphous (70%): m.p.: 177–180°C. IR (KBr) ν_{maks} (cm^{-1}): 3361, 3315 (NH), 1678 (C=O), 1616, 1556, 1519, 1487 (C=N, C=C), 1159 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 3.24 (3H, s, ind. N-CH_3); 7.27 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.48 (1H, ddd, J: 8.6; 2.4; 0.8 Hz, ind. C_6-H); 7.63–7.70 (2H, m, phen. $C_{4,6}$-H); 7.80 (1H, d, J: 1.6 Hz, ind. C_4-H); 7.96–7.99 (1H, m, phen. C_5-H); 8.03 (1H, s, phen. C_2-H); 11.05 (1H, s, N_4-H); 12.68 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 26.42 (ind. N-CH_3); 111.63 (ind. C_7); 114.70 (ind. C_4); 120.68 (q, J: 256.2 Hz, OCF_3); 121.06 (ind. C_{3a}); 122.49 (q, J: 3.8 Hz, phen. C_2); 123.18 (q, J: 3.9 Hz, phen. C_4); 124.42 (q, J: 272.4 Hz, CF_3); 124.73 (ind. C_6); 129.64 (q, J: 31.7 Hz, phen. C_3); 130.08 (phen. C_6); 130.10 (phen. C_5); 131.31 (ind. C_3); 139.55 (phen. C_1); 143.16 (ind. C_{7a}); 144.48 (q, J: 2.0 Hz, ind. C_5); 161.39 (ind. C_2); 177.00 (CS). MS (ESI (+)) $C_{18}H_{13}F_6N_4O_2S$ $[M+H]^+$: 463.37629; Found $[M+H]^+$: 463.40. Anal. Calcd for $C_{18}H_{12}F_6N_4O_2S$: C, 46.76; H, 2.62; N, 12.12; S, 6.93; Found: C, 46.47; H, 2.62; N, 12.09; S, 7.01.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-trifluoromethylphenyl)-thiosemicarbazone] (**95**): Orange crystals (75%): m.p.: 221–223°C. IR (KBr) ν_{maks} (cm^{-1}): 3321, 3226 (NH), 1687 (C=O), 1622, 1602, 1539, 1494 (C=N, C=C), 1141 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 3.26 (3H, s, ind. N-CH_3); 7.27 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.48 (1H, ddd, J: 8.6; 2.4; 0.8 Hz, ind. C_6-H); 7.78–7.82 (3H, m, ind. C_4-H, phen. $C_{2,6}$-H); 7.91 (2H, d, J: 8.2 Hz, phen. $C_{3,5}$-H); 11.06 (1H, s, N_4-H); 12.70 (1H, s, N_2-H). ^{13}C NMR (Decoupled, HSQC-2D, DMSO-d_6/100 MHz) δ (ppm): 25.93 (ind. N-CH_3); 111.12 (ind. C_7); 114.28 (ind. C_4); 120.21 (q, J: 255.5 Hz, OCF_3); 120.55 (ind. C_{3a}); 124.17 (q, J: 272.3 Hz, CF_3); 124.25 (ind. C_6); 125.53 (q, J: 3.9 Hz, phen. $C_{3,5}$); 125.68 (phen. $C_{2,6}$); 126.16 (q, J: 32.3 Hz, phen. C_4); 130.88 (ind. C_3); 141.94 (phen. C_1); 142.68 (ind. C_{7a}); 144.00 (q, J: 1.9 Hz, ind. C_5); 160.90 (ind. C_2); 176.31 (CS). Anal. Calcd for $C_{18}H_{12}F_6N_4O_2S$: C, 46.76; H, 2.62; N, 12.12; S, 6.93; Found: C, 46.72; H, 2.64; N, 12.27; S, 6.88.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-ethylphenyl)thiosemicarbazone] (**96**): Yellow crystals (91%): m.p.: 217–219°C. IR (KBr) ν_{maks} (cm^{-1}): 3334, 3209 (NH), 1683 (C=O), 1618, 1589, 1521, 1506 (C=N, C=C), 1163 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.19 (3H, t, J: 7.4 Hz, $CH_2\underline{CH_3}$); 2.62 (2H, q, J: 7.4 Hz, $\underline{CH_2}CH_3$); 3.24 (3H, s, ind. N-CH_3); 7.25 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.26 (2H, d, J: 8.6 Hz, phen. $C_{3,5}$-H); 7.45–7.47 (1H, m, ind. C_6-H); 7.46 (2H, d, J: 8.2 Hz, phen. $C_{2,6}$-H); 7.82 (1H, d, J: 1.5 Hz, ind. C_4-H); 10.85 (1H, s, N_4-H); 12.55 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 16.07 (CH_2CH_3); 26.41 (ind. N-CH_3); 28.22 (CH_2CH_3); 111.51 (ind. C_7); 114.66 (ind. C_4); 120.68 (q, J: 256.2 Hz, OCF_3); 121.23 (ind. C_{3a}); 124.46 (ind. C_6); 126.24 (phen. $C_{2,6}$); 128.21 (phen. $C_{3,5}$); 130.64 (ind. C_3); 136.36 (phen. C_1); 142.45 (phen. C_4); 142.98 (ind. C_{7a}); 144.47 (q, J: 1.9 Hz, ind. C_5); 161.38 (ind. C_2); 176.78 (CS). MS (ESI (+)) $C_{19}H_{18}F_3N_4O_2S$ $[M+H]^+$: 423.43148; Found $[M+H]^+$: 423.40. Anal. Calcd for $C_{19}H_{17}F_3N_4O_2S$: C, 54.02; H, 4.06; N, 13.26; S, 7.59; Found: C, 54.08; H, 4.09; N, 13.26; S, 7.76.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-tert-butylphenyl)thiosemicarbazone] (**97**): Bright yellow-orange crystals (74%): m.p.: 240–241°C. IR (KBr) ν_{maks} (cm^{-1}): 3319, 3244 (NH), 1687 (C=O), 1622, 1597, 1539, 1489 (C=N, C=C), 1153 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.29 (9H, s, $C(CH_3)_3$); 3.24 (3H, s, ind. N-CH_3); 7.25 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.43 (2H, d, J: 8.6 Hz, phen. $C_{3,5}$-H); 7.44–7.48 (1H, m, ind. C_6-H); 7.49 (2H, d, J: 8.6 Hz, phen. $C_{2,6}$-H); 7.81 (1H, d, J: 1.2 Hz, ind. C_4-H); 10.82 (1H, s, N_4-H); 12.55 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 25.90 (ind. N-CH_3); 31.11 ($C(CH_3)_3$); 34.27 ($C(CH_3)_3$); 111.01 (ind. C_7); 114.15 (ind. C_4); 120.18 (q, J: 256.2 Hz, OCF_3); 120.73 (ind. C_{3a}); 123.95 (ind. C_6); 125.14 (phen. $C_{2,6}$); 125.36 (phen. $C_{3,5}$); 130.19 (ind. C_3); 135.63 (phen. C_1); 142.47 (ind. C_{7a}); 143.98 (q, J: 1.9 Hz, ind. C_5); 143.80 (phen. C_4); 160.87 (ind. C_2); 176.20 (CS). Anal. Calcd for $C_{21}H_{21}F_3N_4O_2S$: C, 55.99; H, 4.70; N, 12.44; S, 7.12; Found: C, 56.11; H, 4.85; N, 12.58; S, 7.24.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-methoxyphenyl)thiosemicarbazone] (**98**): Yellow amorphous (94%): m.p.: 174–175°C. IR (KBr) ν_{maks} (cm^{-1}): 3248, 3209 (NH), 1697 (C=O), 1618, 1598, 1552, 1489 (C=N, C=C), 1166 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 3.25 (3H, s, ind. N-CH_3); 3.79 (3H, s, OCH_3); 6.87 (1H, dd, J: 8.2; 2.7 Hz, phen. C_4-H); 7.19–7.22 (1H, m, phen. C_6-H); 7.25 (1H, dd, J: 2.3; 2.0 Hz, phen. C_2-H); 7.26 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.35 (1H, dd, J: 8.3; 8.2 Hz, phen. C_5-H); 7.47 (1H, dd, J: 8.6; 2.6 Hz, ind. C_6-H); 7.85 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.88 (1H, s, N_4-H); 12.60 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 25.93 (ind. N-CH_3); 55.26 (OCH_3); 111.04 (ind. C_7); 111.36 (phen. C_2); 111.81 (phen. C_4); 114.28 (ind. C_4); 117.81 (phen. C_6); 120.21 (q, J: 255.5 Hz, OCF_3); 120.71 (ind. C_{3a}); 124.04 (ind. C_6); 129.22 (phen. C_5); 130.30 (ind. C_3); 139.33 (phen. C_1); 142.54 (ind. C_{7a}); 144.00 (q, J: 1.9 Hz, ind. C_5); 159.25 (phen. C_3); 160.91 (ind. C_2); 176.11 (CS). LC-MS (ESI (+)) $C_{18}H_{16}F_3N_4O_3S$ $[M+H]^+$: 425.404301; Found $[M+H]^+$: 425.00. Anal. Calcd for $C_{18}H_{15}F_3N_4O_3S.{}^1/_2H_2O$: C, 49.88; H, 3.95; N, 12.92; S, 7.39; Found: C, 50.20; H, 3.82; N, 13.04; S, 7.57.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-methylthiophenyl)thiosemicarbazone] (**100**): Orange-red crystals (94%): m.p.: 207–208°C. IR (KBr) ν_{maks} (cm^{-1}): 3315, 3230 (NH), 1685 (C=O), 1620, 1587, 1537, 1492 (C=N, C=C), 1141 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 2.50 (3H, s, SCH_3); 3.26 (3H, s, ind. N-CH_3); 7.27 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.33 (2H, d, J: 8.9 Hz,

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phen. $C_{3,5}$-H); 7.48 (1H, dd, J: 8.6; 2.7 Hz, ind. C_6-H); 7.55 (2H, d, J: 8.6 Hz, phen. $C_{2,6}$-H); 7.83 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.88 (1H, s, N_4-H); 12.59 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 14.87 (SCH_3); 25.93 (ind. N-CH_3); 111.05 (ind. C_7); 114.20 (ind. C_4); 120.20 (q, J: 256.2 Hz, OCF_3); 120.70 (ind. C_{3a}); 124.03 (ind. C_6); 125.86 (phen. $C_{3,5}$); 126.31 (phen. $C_{2,6}$); 130.31 (ind. C_3); 135.27 (phen. C_1); 136.01 (phen. C_4); 142.52 (ind. C_{7a}); 144.00 (q, J: 1.9 Hz, ind. C_5); 160.90 (ind. C_2); 176.27 (CS). LC-MS (ESI (+)) $C_{18}H_{16}F_3N_4O_2S_2$ $[M+H]^+$: 441.469901; Found $[M+H]^+$: 441.00. Anal. Calcd for $C_{18}H_{15}F_3N_4O_2S_2$: C, 49.08; H, 3.43; N, 12.72; S, 14.56; Found: C, 49.02; H, 3.36; N, 12.84; S, 14.35.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-trifluoromethoxyphenyl)-thiosemicarbazone] (**101**): Light orange powder (96%): m.p.: 201–202°C. IR (KBr) ν_{maks} (cm^{-1}): 3317, 3263 (NH), 1685 (C=O), 1622, 1610, 1552, 1510 (C=N, C=C), 1141 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 3.24 (3H, s, ind. N-CH_3); 7.26 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.44 (2H, d, J: 9.0 Hz, phen. $C_{3,5}$-H); 7.45 (1H, dd, J: 8.6; 2.7 Hz, ind. C_6-H); 7.72 (2H, d, J: 9.0 Hz, phen. $C_{2,6}$-H); 7.79 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.96 (1H, s, N_4-H); 12.63 (1H, s, N_2-H). ^{13}C NMR (Decoupled, DMSO-d_6/100 MHz) δ (ppm): 26.40 (ind. N-CH_3); 111.58 (ind. C_7); 114.63 (ind. C_4); 120.53 (q, J: 256.4 Hz, phen. OCF_3); 120.65 (q, J: 256.6 Hz, ind. OCF_3); 121.09 (ind. C_{3a}); 121.62 (phen. $C_{3,5}$); 124.64 (ind. C_6); 128.08 (phen. $C_{2,6}$); 131.11 (ind. C_3); 137.85 (phen. C_1); 143.09 (ind. C_{7a}); 144.44 (q, J: 2.3 Hz, ind. C_5); 146.47 (q, J: 2.3 Hz, phen. C_4); 161.36 (ind. C_2); 177.01 (CS). MS (ESI (+)) $C_{18}H_{13}F_6N_4O_3S$ $[M+H]^+$: 479.60671; Found $[M+H]^+$: 479.66. Anal. Calcd for $C_{18}H_{12}F_6N_4O_3S$: C, 45.19; H, 2.53; N, 11.71; S, 6.70; Found: C, 45.18; H, 2.55; N, 11.71; S, 6.61.

1-Methyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-fluorophenyl)thiosemicarbazone] (**102**): Bright orange crystals (80%): m.p.: 203–204°C. IR (KBr) ν_{maks} (cm^{-1}): 3321, 3238 (NH), 1687 (C=O), 1612, 1544, 1492 (C=N, C=C), 1145 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 3.24 (3H, s, ind. N-CH_3); 7.11–7.17 (1H, m, phen. C_4-H); 7.26 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.44–7.49 (3H, m, ind. C_6-H, phen. $C_{5,6}$-H); 7.59, 7.60 (1H, 2dd, J: 11.6, 1.6; 11.3, 1.6 Hz, phen. C_2-H); 7.81 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.94 (1H, s, N_4-H); 12.64 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 26.41 (ind. N-CH_3); 111.58 (ind. C_7); 112.98 (d, J: 23.0 Hz, phen. C_2); 113.42 (d, J: 20.1 Hz, phen. C_4); 114.75 (ind. C_4); 120.68 (q, J: 256.2 Hz, OCF_3); 121.09 (ind. C_{3a}); 121.99 (phen. C_6); 124.66 (ind. C_6); 130.48 (d, J: 9.7 Hz, phen. C_5); 131.13 (ind. C_3); 140.39 (d, J: 11.6 Hz, phen. C_1); 143.12 (ind. C_{7a}); 144.48 (q, J: 2.0 Hz, ind. C_5); 161.39 (ind. C_2); 162.11 (d, J: 242.7 Hz, phen. C_3); 176.72 (CS). MS (ESI (+)) $C_{17}H_{13}F_4N_4O_2S$ $[M+H]^+$: 413.36878; Found $[M+H]^+$: 413.40. Anal. Calcd for $C_{17}H_{12}F_4N_4O_2S$: C, 49.52; H, 2.93; N, 13.59; S, 7.78; Found: C, 49.30; H, 2.96; N, 13.51; S, 7.78.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-methylphenyl)thiosemicarbazone] (**103**): Orange crystals (85%): m.p.: 193–194°C. IR (KBr) ν_{maks} (cm^{-1}): 3257 (NH), 1695 (C=O), 1622, 1610, 1593, 1541 (C=N, C=C), 1161 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.20 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 2.33 (3H, s, CH_3); 3.80 (2H, q, J: 7.4 Hz, ind. N-$\underline{CH_2}CH_3$); 7.10 (1H, d, J: 7.4 Hz, phen. C_4-H); 7.29–7.32 (1H, m, phen. C_5-H); 7.31 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.37–7.42 (2H, m, phen. $C_{2,6}$-H); 7.45 (1H, dd, J: 8.6; 2.8 Hz, ind. C_6-H); 7.85 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.84 (1H, s, N_4-H); 12.57 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 13.01 (ind. N-CH_2CH_3); 21.37 (CH_3); 34.77 (ind. N-CH_2CH_3); 111.56 (ind. C_7); 114.92 (ind. C_4); 120.68 (q, J: 256.0 Hz, OCF_3); 121.43 (ind. C_{3a}); 123.35 (phen. C_6); 124.50 (ind. C_6); 126.66 (phen. C_2); 127.44 (phen. C_4); 128.75 (phen. C_5); 130.74 (ind. C_3); 138.33 (phen. C_1); 138.60 (phen. C_3); 141.96 (ind. C_{7a}); 144.40 (q, J: 1.9 Hz, ind. C_5); 161.03 (ind. C_2); 176.70 (CS). MS (ESI (+)) $C_{19}H_{18}F_3N_4O_2S$ $[M+H]^+$: 423.43148; Found $[M+H]^+$: 423.40. Anal. Calcd for $C_{19}H_{17}F_3N_4O_2S$: C, 54.02; H, 4.06; N, 13.26; S, 7.59; Found: C, 53.88; H, 4.02; N, 13.16; S, 7.60.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-methylphenyl)thiosemicarbazone] (**104**): Yellow powder (81%): m.p.: 175–176°C. IR (KBr) ν_{maks} (cm^{-1}): 3273, 3219 (NH), 1697 (C=O), 1624, 1591, 1527 (C=N, C=C), 1165 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.21 (3H, t, J: 7.0 Hz, ind. N-$CH_2\underline{CH_3}$); 2.32 (3H, s, CH_3); 3.80 (2H, q, J: 7.0 Hz, ind. N-$\underline{CH_2}CH_3$); 7.22 (2H, d, J: 8.2 Hz, phen. $C_{3,5}$-H); 7.29 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.40–7.44 (1H, m, ind. C_6-H); 7.46 (2H, d, J: 8.2 Hz, phen. $C_{2,6}$-H); 7.83 (1H, s, ind. C_4-H); 10.80 (1H, s, N_4-H); 12.57 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 12.47 (ind. N-CH_2CH_3); 20.57 (CH_3); 34.25 (ind. N-CH_2CH_3); 111.01 (ind. C_7); 114.35 (ind. C_4); 120.16 (q, J: 256.2 Hz, OCF_3); 120.92 (ind. C_{3a}); 123.91 (ind. C_6); 125.61 (phen. $C_{2,6}$); 128.86 (phen. $C_{3,5}$); 130.13 (ind. C_3); 135.57 (phen. C_4); 135.66 (phen. C_1); 141.39 (ind. C_{7a}); 143.87 (q, J: 1.9 Hz, ind. C_5); 160.49 (ind. C_2); 176.30 (CS). Anal. Calcd for $C_{19}H_{17}F_3N_4O_2S$: C, 54.02; H, 4.06; N, 13.26; S, 7.59; Found: C, 53.63; H, 4.31; N, 13.34; S, 7.76.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-trifluoromethylphenyl)-thiosemicarbazone] (**105**): Orange crystals (73%): m.p.: 162–163°C. IR (KBr) ν_{maks} (cm^{-1}): 3292, 3226 (NH), 1693 (C=O), 1622, 1600, 1556, 1487 (C=N, C=C), 1165 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.20 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 3.80 (2H, q, J: 7.4 Hz, ind. N-$\underline{CH_2}CH_3$); 7.32 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.46 (1H, dd, J: 8.6; 2.0 Hz, ind. C_6-H); 7.62-7.69 (2H, m, phen. $C_{4,6}$-H); 7.80 (1H, d, J: 1.6 Hz, ind. C_4-H); 7.96–7.99 (1H, m, phen. C_5-H); 8.02 (1H, s, phen. C_2-H); 11.04 (1H, s, N_4-H); 12.70 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 12.46 (ind. N-CH_2CH_3); 34.29 (ind. N-CH_2CH_3); 111.16 (ind. C_7); 114.39 (ind. C_4); 120.16 (q, J: 256.2 Hz, OCF_3); 120.75 (ind. C_{3a}); 121.92 (q, J: 3.9 Hz, phen. C_2); 122.63 (q, J: 3.9 Hz, phen. C_4); 123.90 (q, J: 272.3 Hz, CF_3); 124.22 (ind. C_6); 129.12 (q, J: 31.6 Hz, phen. C_3); 129.50 (q, J: 1.3 Hz, phen. C_6); 129.58 (phen. C_5); 130.86 (ind. C_3); 139.04 (phen. C_1); 141.61 (ind. C_{7a}); 143.88 (q, J: 1.9 Hz, ind. C_5); 160.53 (ind. C_2); 176.47 (CS). Anal. Calcd for $C_{19}H_{14}F_6N_4O_2S.{}^1/_2H_2O$: C, 47.01; H, 3.32; N, 11.54; S, 6.60 Found: C, 46.89; H, 2.92; N, 11.92; S, 6.77.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-methoxyphenyl)thiosemicarbazone] (**106**): Yellow amorphous (84%): m.p.: 183–184°C. IR (KBr) ν_{maks} (cm^{-1}): 3290, 3215 (NH), 1697 (C=O), 1620, 1608, 1597, 1552 (C=N, C=C), 1165 (C=S). ^{1}H

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NMR (DMSO-d_6/400 MHz) δ (ppm): 1.21 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 3.78 (3H, s, OCH_3); 3.81 (2H, q, J: 7.4 Hz, ind. N-$\underline{CH_2}CH_3$); 6.86 (1H, dd, J: 8.2; 2.3 Hz, phen. C_4-H); 7.18-7.21 (1H, m, phen. C_6-H); 7.26 (1H, dd, J: 2.4; 2.0 Hz, phen. C_2-H); 7.32 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.34 (1H, dd, J: 8.2; 7.8 Hz, phen. C_5-H); 7.45 (1H, dd, J: 8.6; 2.0 Hz, ind. C_6-H); 7.85 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.86 (1H, s, N_4-H); 12.61 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 13.01 (ind. N-CH_2CH_3); 34.77 (ind. N-CH_2CH_3); 55.71 (OCH_3); 111.57 (ind. C_7); 111.81 (phen. C_2); 112.28 (phen. C_4); 114.96 (ind. C_4); 118.26 (phen. C_6); 120.68 (q, J: 256.2 Hz, OCF_3); 121.39 (ind. C_{3a}); 124.54 (ind. C_6); 129.69 (phen. C_5); 130.84 (ind. C_3); 139.81 (phen. C_1); 141.99 (ind. C_{7a}); 144.40 (q, J: 1.9 Hz, ind. C_5); 159.72 (phen. C_3); 161.04 (ind. C_2); 176.57 (CS). MS (ESI (+)) $C_{19}H_{18}F_3N_4O_3S$ $[M+H]^+$: 439.43088; Found $[M+H]^+$: 439.40. Anal. Calcd for $C_{19}H_{17}F_3N_4O_3S$: C, 52.05; H, 3.91; N, 12.78; S, 7.31; Found: C, 51.70; H, 3.95; N, 12.71; S, 7.41.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-methylthiophenyl)thiosemicarbazone] (**107**): Yellow amorphous (93%): m.p.: 170–173°C. IR (KBr) ν_{maks} (cm^{-1}): 3304, 3211 (NH), 1687 (C=O), 1622, 1585, 1537, 1516 (C=N, C=C), 1163 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.20 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 2.49 (3H, s, SCH_3); 3.80 (2H, q, J: 7.4 Hz, ind. N-$\underline{CH_2}CH_3$); 7.31 (2H, d, J: 8.6 Hz, phen. $C_{3,5}$-H); 7.31 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.44 (1H, dd, J: 8.6; 2.4 Hz, ind. C_6-H); 7.53 (2H, d, J: 8.6 Hz, phen. $C_{2,6}$-H); 7.82 (1H, d, J: 1.2 Hz, ind. C_4-H); 10.87 (1H, s, N_4-H); 12.59 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 12.47 (ind. N-CH_2CH_3); 14.77 (SCH_3); 34.22 (ind. N-CH_2CH_3); 111.04 (ind. C_7); 114.34 (ind. C_4); 120.13 (q, J: 255.5 Hz, OCF_3); 120.85 (ind. C_{3a}); 123.99 (ind. C_6); 125.76 (phen. $C_{3,5}$); 126.22 (phen. $C_{2,6}$); 130.28 (ind. C_3); 135.18 (phen. C_1); 135.92 (phen. C_4); 141.42 (ind. C_{7a}); 143.82 (q, J: 1.9 Hz, ind. C_5); 160.47 (ind. C_2); 176.16 (CS). Anal. Calcd for $C_{19}H_{17}F_3N_4O_2S_2$: C, 50.21; H, 3.77; N, 12.33; S, 14.11; Found: C, 50.44; H, 3.78; N, 12.50; S, 14.11.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-fluorophenyl)thiosemicarbazone] (**108**): Yellow powder (80%): m.p.: 166–167°C. IR (KBr) ν_{maks} (cm^{-1}): 3263, 3213 (NH), 1693 (C=O), 1625, 1600, 1548, 1533 (C=N, C=C), 1174 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.21 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 3.81 (2H, q, J: 7.4 Hz, ind. N-$\underline{CH_2}CH_3$); 7.11-7.17 (1H, m, phen. C_4-H); 7.33 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.44-7.50 (3H, m, ind. C_6-H, phen. $C_{5,6}$-H); 7.60, 7.61 (1H, 2dd, J: 10.9, 1.9; 10.6, 1.6 Hz, phen. C_2-H); 7.83 (1H, d, J: 1.5 Hz, ind. C_4-H); 10.94 (1H, s, N_4-H); 12.66 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 12.47 (ind. N-CH_2CH_3); 34.26 (ind. N-CH_2CH_3); 111.10 (ind. C_7); 112.43 (d, J: 24.5 Hz, phen. C_2); 112.87 (d, J: 21.3 Hz, phen. C_4); 114.43 (ind. C_4); 120.14 (q, J: 256.2 Hz, OCF_3); 120.76 (ind. C_{3a}); 121.43 (d, J: 2.6 Hz, phen. C_6); 124.15 (ind. C_6); 129.95 (d, J: 9.7 Hz, phen. C_5); 130.66 (ind. C_3); 139.86 (d, J: 11.0 Hz, phen. C_1); 141.55 (ind. C_{7a}); 143.86 (q, J: 1.9 Hz, ind. C_5); 160.50 (ind. C_2); 161.58 (d, J: 242.6 Hz, phen. C_3); 176.15 (CS). Anal. Calcd for $C_{18}H_{14}F_4N_4O_2S$: C, 50.70; H, 3.31; N, 13.14; S, 7.52; Found: C, 50.52; H, 3.25; N, 13.13; S, 7.57.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-fluorophenyl)thiosemicarbazone] (**109**): Yellow crystals (84%): m.p.: 172°C. IR (KBr) ν_{maks} (cm^{-1}): 3307, 3197 (NH), 1678 (C=O), 1616, 1519, 1471 (C=N, C=C), 1172 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.21 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 3.81 (2H, q, J: 7.5 Hz, ind. N-$\underline{CH_2}CH_3$); 7.28 (2H, dd, J: 9.1; 8.5 Hz, phen. $C_{3,5}$-H); 7.32 (1H, d, J: 8.5 Hz, ind. C_7-H); 7.45 (1H, ddd, J: 8.6; 2.8; 0.8 Hz, ind. C_6-H); 7.59 (2H, dd, J: 9.0; 5.1 Hz, phen. $C_{2,6}$-H); 7.81 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.91 (1H, s, N_4-H); 12.60 (1H, s, N_2-H). ^{13}C NMR (Decoupled APT, DMSO-d_6/100 MHz) δ (ppm): 12.47 (ind. N-CH_2CH_3); 34.25 (ind. N-CH_2CH_3); 111.07 (ind. C_7); 114.29 (ind. C_4); 115.17 (d, J: 22.6 Hz, phen. $C_{3,5}$); 120.15 (q, J: 255.5 Hz, OCF_3); 120.85 (ind. C_{3a}); 124.04 (ind. C_6); 128.06 (d, J: 9.0 Hz, phen. $C_{2,6}$); 130.42 (ind. C_3); 134.55 (d, J: 3.2 Hz, phen. C_1); 141.47 (ind. C_{7a}); 143.86 (q, J: 1.9 Hz, ind. C_5); 160.10 (d, J: 243.3 Hz, phen. C_4); 160.48 (ind. C_2); 176.71 (CS). Anal. Calcd for $C_{18}H_{14}F_4N_4O_2S$: C, 50.70; H, 3.31; N, 13.14; S, 7.52; Found: C, 50.34; H, 3.69; N, 13.03; S, 7.94.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-chlorophenyl)thiosemicarbazone] (**110**): Yellow powder (79%): m.p.: 198–199°C. IR (KBr) ν_{maks} (cm^{-1}): 3248, 3211 (NH), 1693 (C=O), 1624, 1583, 1541, 1527 (C=N, C=C), 1176 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.21 (3H, t, J: 7.4 Hz, ind. N-$CH_2\underline{CH_3}$); 3.81 (2H, q, J: 7.4 Hz, ind. N-$\underline{CH_2}CH_3$); 7.33 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.35 (1H, ddd, J: 8.2; 2.0; 0.8 Hz, phen. C_4-H); 7.46 (1H, dd, J: 8.2; 7.8 Hz, phen. C_5-H); 7.46 (1H, dd, J: 8.6; 2.8 Hz, ind. C_6-H); 7.63 (1H, ddd, J: 7.8; 2.0; 0.8 Hz, phen. C_6-H); 7.76 (1H, dd, J: 2.0; 2.0 Hz, phen. C_2-H); 7.82 (1H, d, J: 1.5 Hz, ind. C_4-H); 10.94 (1H, s, N_4-H); 12.66 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 13.00 (ind. N-CH_2CH_3); 34.80 (ind. N-CH_2CH_3); 111.65 (ind. C_7); 114.94 (ind. C_4); 120.68 (q, J: 256.2 Hz, OCF_3); 121.30 (ind. C_{3a}); 124.68 (ind. C_6, phen. C_6); 125.69 (phen. C_2); 126.54 (phen. C_4); 130.53 (phen. C_5); 131.26 (ind. C_3); 133.01 (phen. C_3); 140.18 (phen. C_1); 142.10 (ind. C_{7a}); 144.40 (q, J: 1.9 Hz, ind. C_5); 161.04 (ind. C_2); 176.78 (CS). MS (ESI (+)) $C_{18}H_{15}ClF_3N_4O_2S$ $[M+H]^+$: 443.84996; Found $[M+H]^+$: 443.40, 445.30. Anal. Calcd for $C_{18}H_{14}ClF_3N_4O_2S$: C, 48.82; H, 3.19; N, 12.65; S, 7.24; Found: C, 48.58; H, 3.16; N, 12.70; S, 7.25.

1-Ethyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-chlorophenyl)thiosemicarbazone] (**111**): Bright orange crystals (79%): m.p.: 190°C. IR (KBr) ν_{maks} (cm^{-1}): 3290, 3217 (NH), 1691 (C=O), 1622, 1589, 1529, 1485 (C=N, C=C), 1161 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.21 (3H, t, J: 7.0 Hz, ind. N-$CH_2\underline{CH_3}$); 3.81 (2H, q, J: 7.0 Hz, ind. N-$\underline{CH_2}CH_3$); 7.32 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.46 (1H, ddd, J: 8.6; 2.3; 0.8 Hz, ind. C_6-H); 7.49 (2H, d, J: 9.0 Hz, phen. $C_{3,5}$-H); 7.64 (2H, d, J: 8.6 Hz, phen. $C_{2,6}$-H); 7.82 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.93 (1H, s, N_4-H); 12.64 (1H, s, N_2-H). ^{13}C NMR (Decoupled, DMSO-d_6/100 MHz) δ (ppm): 12.50 (ind. N-CH_2CH_3); 34.30 (ind. N-CH_2CH_3); 111.13 (ind. C_7); 114.39 (ind. C_4); 120.18 (q, J: 256.2 Hz, OCF_3); 120.83 (ind. C_{3a}); 124.15 (ind. C_6); 127.42 (phen. $C_{2,6}$); 128.38 (phen. $C_{3,5}$); 130.33 (phen. C_4); 130.61 (ind. C_3); 137.22 (phen. C_1); 141.55 (ind. C_{7a}); 143.90 (q, J: 1.9 Hz, ind. C_5); 160.53 (ind. C_2);

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176.39 (CS). MS (ESI (+)) $C_{18}H_{15}ClF_3N_4O_2S$ $[M+H]^+$: 443.84996; Found $[M+H]^+$: 443.67, 445.43. Anal. Calcd for $C_{18}H_{14}ClF_3N_4O_2S$: C, 48.82; H, 3.19; N, 12.65; S, 7.24; Found: C, 48.59; H, 3.15; N, 12.66; S, 7.27.

1-Benzyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazone) (**112**): Yellow powder (81%): m.p.: 155–157°C. IR (KBr): 3329 (NH), 1685 (C=O), 1620, 1598, 1546, 1521 (C=N, C=C), 1182 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 5.02 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.13 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.27-7.34 (2H, m, benz. C_4-H, phen. C_4-H); 7.35 (2H, dd, J: 7.4; 7.0 Hz, benz. $C_{3,5}$-H); 7.38–7.42 (1H, m, ind. C_6-H); 7.41 (2H, d, J: 7.0 Hz, benz. $C_{2,6}$-H); 7.45 (2H, t, J: 7.8 Hz, phen. $C_{3,5}$-H); 7.60 (2H, d, J: 7.8 Hz, phen. $C_{2,6}$-H); 7.88 (1H, d, J: 1.5 Hz, ind. C_4-H); 10.96 (1H, s, N_4-H); 12.60 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 43.18 (ind. N-$CH_2C_6H_5$); 112.00 (ind. C_7); 114.89 (ind. C_4); 120.64 (q, J: 256.2 Hz, OCF_3); 121.55 (ind. C_{3a}); 124.46 (ind. C_6); 126.37 (phen. $C_{2,6}$); 126.88 (phen. C_4); 127.93 (benz. $C_{2,6}$); 128.17 (benz. C_4); 128.98 (phen. $C_{3,5}$); 129.21 (benz. $C_{3,5}$); 130.59 (ind. C_3); 135.89 (benz. C_1); 138.73 (phen. C_1); 141.94 (ind. C_{7a}); 144.55 (q, J: 1.9 Hz, ind. C_5); 161.43 (ind. C_2); 176.87 (CS). MS (ESI (+)) $C_{23}H_{18}F_3N_4O_2S$ $[M+H]^+$: 471.47428; Found $[M+H]^+$: 471.60. Anal. Calcd for $C_{23}H_{17}F_3N_4O_2S$: C, 58.72; H, 3.64; N, 11.91; S, 6.82; Found: C, 58.79; H, 3.72; N, 11.95; S, 6.88.

1-Benzyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-methylphenyl)thiosemicarbazone] (**113**): Yellow amorphous (93%): m.p.: 200–203°C. IR (KBr): 3248 (NH), 1697 (C=O), 1620, 1610, 1591, 1539 (C=N, C=C), 1153 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 2.34 (3H, s, CH_3); 5.01 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.09–7.12 (1H, m, phen. C_4-H); 7.11 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.25-7.29 (1H, m, benz. C_4-H); 7.31–7.36 (1H, m, phen. C_5-H); 7.34 (2H, dd, J: 7.4; 7.0 Hz, benz. $C_{3,5}$-H); 7.38–7.43 (5H, m, benz. $C_{2,6}$-H, ind. C_6-H, phen. $C_{2,6}$-H); 7.87 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.88 (1H, s, N_4-H); 12.57 (1H, s, N_2-H). ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz) δ (ppm): 21.38 (CH_3); 43.18 (ind. N-$CH_2C_6H_5$); 111.99 (ind. C_7); 114.91 (ind. C_4); 120.64 (q, J: 256.0 Hz, OCF_3); 121.56 (ind. C_{3a}); 123.40 (phen. C_6); 124.41 (ind. C_6); 126.66 (phen. C_2); 127.48 (phen. C_4); 127.92 (benz. $C_{2,6}$); 128.16 (benz. C_4); 128.78 (phen. C_5); 129.20 (benz. $C_{3,5}$); 130.50 (ind. C_3); 135.89 (benz. C_1); 138.36 (phen. C_1); 138.61 (phen. C_3); 141.93 (ind. C_{7a}); 144.55 (q, J: 1.9 Hz, ind. C_5); 161.42 (ind. C_2); 176.72 (CS). MS (ESI (+)) $C_{24}H_{20}F_3N_4O_2S$ $[M+H]^+$: 485.50086; Found $[M+H]^+$: 485.50. Anal. Calcd for $C_{24}H_{19}F_3N_4O_2S$: C, 59.50; H, 3.95; N, 11.56; S, 6.62; Found: C, 59.26; H, 4.10; N, 11.69; S, 6.87.

1-Benzyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-methylphenyl)thiosemicarbazone] (**114**): Orange amorphous (96%): m.p.: 172–173°C. IR (KBr): 3346, 3219 (NH), 1687 (C=O), 1620, 1583, 1521, 1504 (C=N, C=C), 1149 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 2.33 (3H, s, CH_3); 5.02 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.12 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.24 (2H, d, J: 8.2 Hz, phen. $C_{3,5}$-H); 7.26–7.30 (1H, m, benz. C_4-H); 7.34 (2H, dd, J: 7.4; 7.0 Hz, benz. $C_{3,5}$-H); 7.36–7.40 (1H, m, ind. C_6-H); 7.41 (2H, d, J: 7.0 Hz, benz. $C_{2,6}$-H); 7.46 (2H, d, J: 8.2 Hz, phen. $C_{2,6}$-H); 7.87 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.89 (1H, s, N_4-H); 12.57 (1H, s, N_2-H). ^{13}C NMR (Decoupled DEPT, HMBC-2D, DMSO-d_6/100 MHz) δ (ppm): 21.11 (CH_3); 43.16 (ind. N-$CH_2C_6H_5$); 111.97 (ind. C_7); 114.86 (ind. C_4); 120.63 (q, J: 256.1 Hz, OCF_3); 121.56 (ind. C_{3a}); 124.38 (ind. C_6); 126.20 (phen. $C_{2,6}$); 127.91 (benz. $C_{2,6}$); 128.15 (benz. C_4); 129.19 (benz. $C_{3,5}$); 129.42 (phen. $C_{3,5}$); 130.43 (ind. C_3); 135.90 (benz. C_1); 136.15 (phen. C_4); 136.16 (phen. C_1); 141.89 (ind. C_{7a}); 144.53 (q, J: 2.3 Hz, ind. C_5); 161.41 (ind. C_2); 176.83 (CS). MS (ESI (+)) $C_{24}H_{20}F_3N_4O_2S$ $[M+H]^+$: 485.50086; Found $[M+H]^+$: 485.86. Anal. Calcd for $C_{24}H_{19}F_3N_4O_2S$: C, 59.50; H, 3.95; N, 11.56; S, 6.62; Found: C, 59.17; H, 4.10; N, 11.59; S, 6.67.

1-Benzyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(4-ethylphenyl)thiosemicarbazone] (**115**): Yellow amorphous (84%): m.p.: 156–157°C. IR (KBr): 3346, 3321, 3226 (NH), 1689 (C=O), 1593, 1537, 1521 (C=N, C=C), 1151 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 1.20 (3H, t, J: 7.4 Hz, $CH_2\underline{CH_3}$); 2.63 (2H, q, J: 7.4 Hz, $\underline{CH_2}CH_3$); 5.02 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.12 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.27 (2H, d, J: 8.6 Hz, phen. $C_{3,5}$-H); 7.26–7.30 (1H, m, benz. C_4-H); 7.34 (2H, dd, J: 7.8; 7.0 Hz, benz. $C_{3,5}$-H); 7.37–7.41 (1H, m, ind. C_6-H); 7.40 (2H, d, J: 7.0 Hz, benz. $C_{2,6}$-H); 7.48 (2H, d, J: 8.2 Hz, phen. $C_{2,6}$-H); 7.86 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.90 (1H, s, N_4-H); 12.57 (1H, s, N_2-H). ^{13}C NMR (Decoupled DEPT, DMSO-d_6/100 MHz) δ (ppm): 15.58 (CH_2CH_3); 27.74 (CH_2CH_3); 42.68 (ind. N-$CH_2C_6H_5$); 111.48 (ind. C_7); 114.37 (ind. C_4); 120.14 (q, J: 256.2 Hz, OCF_3); 121.07 (ind. C_{3a}); 123.88 (ind. C_6); 125.75 (phen. $C_{2,6}$); 127.42 (benz. $C_{2,6}$); 127.65 (benz. C_4); 127.72 (phen. $C_{3,5}$); 128.70 (benz. $C_{3,5}$); 129.94 (ind. C_3); 135.40 (benz. C_1); 135.85 (phen. C_1); 141.39 (phen. C_4); 141.98 (ind. C_{7a}); 144.03 (q, J: 2.0 Hz, ind. C_5); 160.92 (ind. C_2); 176.29 (CS). Anal. Calcd for $C_{25}H_{21}F_3N_4O_2S$: C, 60.23; H, 4.25; N, 11.24; S, 6.43; Found: C, 60.46; H, 4.33; N, 11.23; S, 6.51.

1-Benzyl-5-(trifluoromethoxy)−1*H*-indole-2,3-dione 3-[4-(3-fluorophenyl)thiosemicarbazone] (**116**): Yellow powder (89%): m.p.: 189–191°C. IR (KBr): 3344, 3317 (NH), 1685 (C=O), 1622, 1604, 1556, 1496 (C=N, C=C), 1188 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) δ (ppm): 5.01 (2H, s, ind. N-$\underline{CH_2}C_6H_5$); 7.10–7.15 (1H, m, phen. C_4-H); 7.12 (1H, d, J: 8.6 Hz, ind. C_7-H); 7.26–7.30 (1H, m, benz. C_4-H); 7.34 (2H, dd, J: 7.4; 7.1 Hz, benz. $C_{3,5}$-H); 7.37–7.41 (3H, m, benz. $C_{2,6}$-H, ind. C_6-H); 7.44–7.50 (2H, m, phen. $C_{5,6}$-H); 7.60, 7.61 (1H, 2dd, J: 11.4, 2.4; 10.6, 1.6 Hz, phen. C_2-H); 7.85 (1H, d, J: 1.6 Hz, ind. C_4-H); 10.97 (1H, s, N_4-H); 12.65 (1H, s, N_2-H). ^{13}C NMR (Decoupled, HSQC-2D, DMSO-d_6/100 MHz) δ (ppm): 42.70 (ind. N-$CH_2C_6H_5$); 111.55 (ind. C_7); 112.50 (d, J: 24.5 Hz, phen. C_2); 112.96 (d, J: 20.7 Hz, phen. C_4); 114.45 (ind. C_4); 120.14 (q, J: 256.2 Hz, OCF_3); 120.93 (ind. C_{3a}); 121.51 (d, J: 3.2 Hz, phen. C_6); 124.09 (ind. C_6); 127.43 (benz. $C_{2,6}$); 127.67 (benz. C_4); 128.70 (benz. $C_{3,5}$); 130.02 (d, J: 9.0 Hz, phen. C_5); 130.47 (ind. C_3); 135.35 (benz. C_1); 139.89 (d, J: 10.3 Hz, phen. C_1); 141.55 (ind. C_{7a}); 144.06 (q, J: 2.0 Hz, ind. C_5); 160.94 (ind. C_2); 161.63 (d, J: 243.2 Hz, phen. C_3); 176.24 (CS). Anal. Calcd for $C_{23}H_{16}F_4N_4O_2S$: C, 56.55; H, 3.30; N, 11.47; S, 6.56; Found: C, 56.72; H, 3.52; N, 11.48; S, 6.64.

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4.3 | In vitro experiments

4.3.1 | Interleukin-1 receptor (IL-1R) inhibiting assays

HEK-Blue IL-1R cell lines (InvivoGen, cat no. hkb-il1r) with binary cell culture in DMEM (Gibco, cat no. 41965039) containing 10% fetal bovine serum (Gibco, cat no. 10500064) and 1% penicillin/streptomycin (Gibco, cat no. 15140122) culture series were formed. The stock solutions of the compounds were prepared by dissolving in DMSO (Sigma-Aldrich, cat no. D2650) to 10 mM. 3 × 104 HEK-Blue IL-1R cells, stimulated with 1.3 ng/mL hIL-1β (Peprotech, cat no. 200-01B), were treated with various concentrations of novel compounds (9.7 nM–20 µM) and incubated for 24 h at 37°C. As positive control, cells treated with 1.3 ng/mL hIL-1β were used. As viability control, cells were treated with 10% DMSO. 2M diethanolamine solution (Sigma-Aldrich, cat no. D8885) containing 1 mM $MgCl_2$ (Sigma-Aldrich, cat no. 208337) and 0.5 mM $ZnCl_2$ (Sigma-Aldrich, cat no. 208086) was prepared as a colorimetric solution to detect SEAP levels. This solution was mixed with p-nitrophenyl phosphate (Sigma-Aldrich, cat no. 71768) with the final concentration of 1 mg/mL. Separately, 15% CHAPS detergent (Sigma-Aldrich, cat no. C3023) was prepared, and the final concentration of 0.05% CHAPS is prepared with 1X DPBS (Gibco, cat no. 14190144). 20 µL 0.05% CHAPS was mixed with 40 µL of the cell suspension, then 200 µL of the colorimetric solution was added, and allowed to incubate for 1 h at room temperature. After incubation, the absorbance in each well was measured at 405 nm using Biotek Synergy™ H1 Hybrid Multi-Mode Reader. The results from novel compound cells were normalized according to the positive and viability controls, and the concentration-response curves were plotted using Prism v.9 (GraphPad Software Inc.). We calculated the IC_{50} values, expressed as mean ± SD, by drawing concentration-inhibition curves using GraphPad. For each experiment, at least three biological replicates with all compounds and three technical replicates for each experiment set, we prepared freshly made solutions from the powder forms of the compounds. Anakinra, a known inhibitor of IL-1R, was used as a positive control to validate the experiments.

4.3.2 | MTS cell viability test

In the mitochondria of living cells, yellow MTS [3-(4,5-dimethylthiazol-2-yl)−5-(3-carboxymethoxyphenyl)−2-(4-sulfophenyl)−2*H*-tetrazolium bromide)] is reduced to purple formazan that is soluble in cell culture media. Since this reduction occurs only when mitochondrial reductase enzymes are active, the conversion is directly related to viable cell count and metabolic activity. After 40 µL were transferred for use in SEAP, the test plates were used for the MTS assay. Fifteen microliters of MTS reagent (Promega, cat no. G1111) was added to each well, and after 3 h of incubation at 37°C, the absorbance in each well was measured at 490 nm using Biotek Synergy™ H1 Hybrid Multi-Mode Reader. Results from cells which contain novel compounds were normalized according to positive and viability controls, and concentration-response curves were plotted.

4.3.3 | Cyclooxygenase measurements

COX-1 inhibition activity was measured using a COX-1 inhibitor screening assay kit from Cayman Chemical (Ref. 701090). The compounds 78 and 81, and indomethacin (a potent nonselective COX-1/2 inhibitor), as a positive control, were tested in triplicate at 0.3 nM to 30 µM range. All tested compounds were preincubated with ovine COX-1 at 37°C for 10 min before incubation with COX substrate arachidonic acid for another 3 min. Prostanoid standard curve was prepared, and an enzyme immunoassay was performed according to the manufacturer's specifications. A DMSO control and a 100% inhibition control with heat-inactivated COX enzyme were also included for reference. Standard curve linear fit was used to calculate each sample's concentrations. Prostanoid standard values were plotted as logit (B/B_0) versus log concentrations and fitted with nonlinear regression using GraphPad Prism v. 9.0. to calculate IC_{50} values.

ACKNOWLEDGMENTS

The authors thank Dr. Özgecan Kayalar (Koç University, KUTTAM) for his valuable help in COX measurements. Authors gratefully acknowledge the use of the services and facilities of the Koç University Research Center for Translational Medicine (KUTTAM) for in vitro experiments. This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number 1003-215S011).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. Two patent applications were filed concerning the chemicals synthesized in this study (numbers. TR 2016018981 A2 20170821 and PCT/TR 2020/050104) were filed.

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How to cite this article: Ö. Soylu-Eter, Z. Ş. Sevinçli, B. Ersoy, B. Hasanusta, U. Gatfar, N. A. Lack, B. Erman, A. Gül, H. S. Orer, N. Karalı, *Arch. Pharm.* **2023**;*356*:e2300217. https://doi.org/10.1002/ardp.202300217

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