Received: 27 February 2020 | Revised: 31 March 2020 | Accepted: 2 April 2020

DOI: 10.1002/ardp.202000061

FULL PAPER

DPhG ARCH PHARM Archiv der Pharmazie

Synthesis, anti-inflammatory activity, and molecular docking studies of some novel Mannich bases of the 1,3,4-oxadiazole-2(3*H*)-thione scaffold

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Abstract

A series of novel ibuprofen and salicylic acid-based 3,5-disubstituted-1,3,4-oxadiazole-2(3*H*)-thione derivatives was synthesized, and they were evaluated as potential anti-inflammatory agents. Following the structure identification studies employing IR, ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and elemental analysis, the title compounds were tested by cyclooxygenase (COX)-1 and COX-2 inhibition assays concomitant to lipopolysaccharide (LPS)-induced nitric oxide and prostaglandin production prevention experiments. The results indicated that the majority of the compounds displayed either a superior or comparable activity in preventing both LPS-induced NO production and COX-1 activity in comparison to the activities of the reference molecules. Furthermore, docking studies were also performed to reveal possible interactions with the COX enzymes.

KEYWORDS

1,3,4-oxadiazole, anti-inflammatory activity, COX-1/COX-2, LPS-induced NO production, molecular docking study, PGE_2

1 | INTRODUCTION

Inflammation occurs as an immediate body response to tissue and cellular damage caused by pathogens, chemicals, or physical injury. According to causative agents, primary mediators, onset or duration of actions, and severity of outcomes, inflammation is divided into two main groups. Acute inflammation is a short-term action that usually results in healing, in which the infiltration of leukocytes into the damaged region ends up with the removal of the stimulus. However, chronic inflammation is a prolonged, dysregulated and maladaptive action that includes tissue damages. Such long-term inflammation is related to many chronic conditions and diseases, including allergy, atherosclerosis, cancer, arthritis, and autoimmune diseases.[1–3] Unrelated to the origin of its response, the "purpose" of inflammation is to eliminate the source of the disturbance. If abnormal conditions are temporary, the inflamed host is adapted to restore functionality and homeostasis of the tissue. Therefore, these acute inflammatory responses back basal homeostatic set points. Otherwise, sustained inflammatory states shift through different physiological setpoints, as they occur during chronic inflammation.[4]

Steroidal and nonsteroidal anti-inflammatory drugs are used to relieve inflammation, pain, and edema through the inhibition of phospholipase A_2, cyclooxygenases (COXs), and lipoxygenases, respectively. The COX family is responsible for the biosynthesis of prostaglandin H_2, which is the precursor for some of the inflammatory mediators, prostaglandins, prostacyclins, and thromboxanes. Their biological missions are coordinated by two major enzyme isoforms, COX-1 and COX-2. COX-1 is constitutively secreted and located in most tissues to maintain homeostasis, whereas COX-2 is synthesized upon an inflammatory response and evaluated at the site of inflammation. In many cases of anti-inflammatory drug therapy, due to nonselective inhibition of both enzymes, there was the emergence of systemic and local gastrointestinal side effects, thus leading to the requirement of new pharmaceutical approaches to overcome these problems.[5–8]

Nitric oxide (NO), which is secreted by inducible nitric oxide synthase from activated macrophages during the inflammation response, plays an important role in cartilage catabolism through the inflammation. It interacts with inducible COX in pathologic conditions and enhances the formation of prostaglandin E_2 (PGE_2) prostanoid.[9] PGE_2 is involved in many processes leading to the general symptoms of inflammation: redness, swelling, and pain. Redness and edema emerge from the increased blood flow into the inflamed tissue dependent on PGE_2-mediated arterial dilatation and increased microvascular permeability. Such events mean that PGE_2 inhibition also plays a crucial role in the removal of inflammation, thus, eventually contributing to tissue remodeling.[10,11]

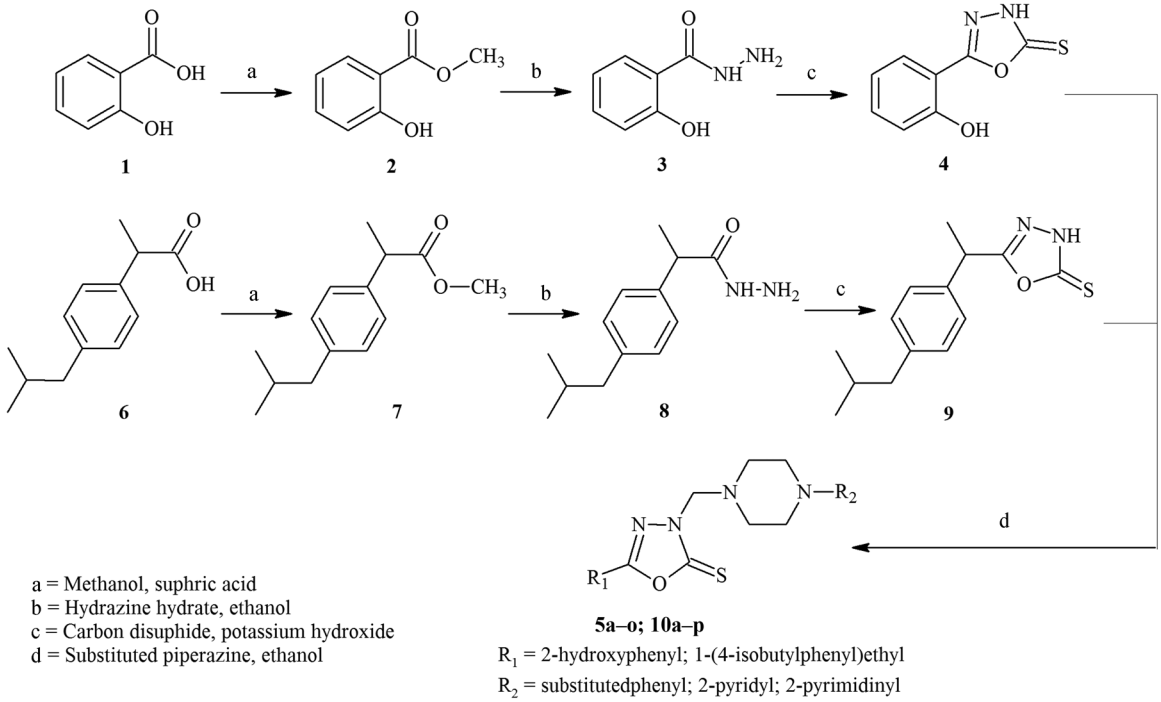
The molecular and conceptual model of inflammation has been continuously evolving to provide new insights to generate novel therapeutic approaches for inflammatory diseases. Particularly, recent studies have aimed to replace the carboxylate functionality of nonsteroidal anti-inflammatory drugs with many types of less acidic heterocyclic bioisosteres, such as 1,3,4-oxadiazole,[12] 1,2,4-triazole,[13,14] and 1,3,4-thiadiazole,[15] to protect the gastric mucosa from free carboxylate moiety.[16] On the basis of this principle, selective COX-2 inhibitors were developed, which commonly have a heterocyclic core and their action on cytoprotective prostaglandins contribute to physiological homeostasis besides their anti-inflammatory actions. However, valdecoxib (Bextra®) and rofecoxib (Vioxx®) presented severe adverse effects on the cardiovascular system, and they have been withdrawn from the market.[17–19]

Motivated by these approaches and based on the crucial heterocyclic skeleton structure of anti-inflammatory compounds, our primary aim was to design new 3,5-disubstituted-1,3,4-oxadiazole-2(*3H*)-thione compounds and clarify their potent responses for fundamental inflammatory enzymes (COX-1, COX-2) and mediators (PGE_2, NO) by using in vitro methods. In addition, a molecular docking analysis was performed to disclose the binding interaction template of the most active inhibitor with COX-1 and COX-2 enzymes.

2 | RESULTS AND DISCUSSION

2.1 | Synthesis

In this study, derivatives of 5-(2-hydroxyphenyl)-3-[(substitutedpiperazine)methyl]-1,3,4-oxadiazole-2(3*H*)-thiones (compounds **5a–o**) and 5-[1-(4-isobutylphenyl)ethyl]-3-[(4-substitutedpiperazine)methyl]-1,3,4-oxadiazole-2(3*H*)-thiones (compounds **10a–p**) were synthesized according to the reaction pathway depicted in Scheme 1. In the first step, carboxylic acid groups of salicylic acid (compound **1**) or ibuprofen (compound **6**) were converted into methyl ester groups (compounds **2**, **7**) in the presence of sulfuric acid and methanol. Subsequently, these esters were reacted with hydrazine hydrate to produce hydrazide derivatives (compounds **3** and **8**). The ring closure of 1,3,4-oxadiazole-2(3*H*)-thione (compounds **4** and **9**) was accomplished via the reaction of hydrazides with carbon disulfide (CS_2) and potassium hydroxide (KOH) in hot alcoholic media.[20,21] Finally, compounds **4** and **9** were treated with different piperazines via Mannich reaction procedure to afford target compounds **5a–o** and **10a–p**. Novel 3,5-disubstituted-1,3,4-oxadiazole-2(3*H*)-thione derivatives were yielded in the range of 32–84% for the salicylic acid



SCHEME 1 The chemical synthesis of compounds **5a–o** and **10a–p**

TABLE 1 Yields of compounds **5a–o** and **10a–p**

Salicylic acid derivatives R_1 = 2-hydroxyphenyl			Ibuprofen derivatives R_1 = 1-(4-isobutylphenyl)ethyl		
Compound	R_2	**Yield (%)**	**Compound**	R_2	**Yield (%)**
5a	Phenyl	84	**10a**	Phenyl	72
5b	4-fluorophenyl	76	**10b**	4-fluorophenyl	69
5c	4-trifluoromethylphenyl	61	**10c**	2-fluorophenyl	40
5d	3-trifluoromethylphenyl	30	**10d**	3-trifluorophenyl	56
5e	4-chlorophenyl	56	**10e**	4-chlorophenyl	54
5f	2-chlorophenyl	32	**10f**	2-chlorophenyl	39
5g	3,4-dichlorophenyl	75	**10g**	3,4-dichlorophenyl	63
5h	2,3-dichlorophenyl	40	**10h**	2,3-dichlorophenyl	52
5i	4-methylphenyl	51	**10i**	4-methylphenyl	29
5j	2,3-dimethylphenyl	66	**10j**	2,3-dimethylphenyl	45
5k	4-methoxyphenyl	32	**10k**	4-methoxyphenyl	47
5l	4-cyanophenyl	55	**10l**	3-methoxyphenyl	32
5m	2-cyanophenyl	56	**10m**	2-methoxyphenyl	41
5n	2-pyridyl	59	**10n**	4-cyanophenyl	50
5o	2-pyrimidinyl	60	**10o**	2-cyanophenyl	52
			10p	2-pyridyl	55

series (**5a–o**) and 29–72% for the ibuprofen series (**10a–p**; Table 1). Particularly, *para*-substituted compounds showed higher yields, compared with *ortho*- and *meta*-substituted ones.

Structures of the synthesized target compounds were confirmed by spectroscopic methods. Basically, the ring closure of 1,3,4-oxadiazole-2(3*H*)-thione was characterized by using IR, with the disappearance of N–H and the emergence of C═N and C═S stretching signals nearly at 3,300, 1,600–1,650, and 1,100–1,250 cm^{-1}, respectively. The signals for C–O stretchings in the range of 1,200–1,350 cm^{-1} were also observed to confirm the ring closure. Additionally, thione and thiol tautomers of 1,3,4-oxadiazole were indicated by weak signals around 1,300 and 2,600 cm^{-1}, respectively. Aromatic C–H stretchings of **5a–o** and **10a–p** were detected between 2,800 and 2,900 cm^{-1}, whereas aromatic C═C stretchings appeared around 1,450 cm^{-1} in both series. Identically, compounds **5a–o** presented a broad signal in the range of 3,304–3,436 cm^{-1} due to the hydroxy group of the salicylic acid moiety. IR data also indicated the loss of N–H stretchings at 3,300 cm^{-1}, proposing the formation of all newly synthesized Mannich bases (**5a–o** and **10a–p**).

Besides Fourier-transform infrared spectroscopy (FT-IR), ^1H nuclear magnetic resonance (NMR) was another indicator for methylene hydrogens between oxadiazole and piperazine moieties. Particularly descriptive singlet signal of methylene protons, found in the range of 4.99–5.10 ppm, represented Mannich bases of target compounds. H_2 and H_6 protons of the piperazine ring were generally observed at about 2.88 ppm, and H_3 and H_5 protons were observed at 2.96–3.28 ppm as triplet signals due to the substituent effect on the phenyl ring. The other protons belonging to substituents of piperazine, aromatic ring protons, and ibuprofen alkyl groups attached to oxadiazole ring were observed at the expected values.

Molecular structures of the final compounds (**5a–o**, **10a–p**) were also supported with ^{13}C NMR spectroscopy. On the basis of carbon nucleus under the influence of a high electronegative environment, aromatic carbon atoms linked with the fifth position of 1,3,4-oxadiazole-2(3*H*)-thione ring appeared at a more downfield region in both compound series. A similar reasoning also explains the peak values of thione (C═S) and imine (C═N) groups, which appeared at nearly 177 and 155 ppm. In salicylic acid derivatives (**5a–o**), chemical shift values of C_2 carbon directly linked to hydroxyl group on phenyl ring appeared in the range of 155–153 ppm. Other carbon atoms of two phenyl rings were sequenced in a decreasing order of their values, based on their electronic environments.

2.2 | Biological evaluation

2.2.1 | Cell viability

To determine safe and non-toxic concentrations of each compound, cytotoxicity testing was carried out before in vitro anti-inflammatory activity screening. Safe doses of compounds were

TABLE 2 Cell viability and nitric oxide (NO) production inhibitory activities of compounds **5a–5o** and **10a–10p**

Compound[a]	Cell viability (%)[b]	NO inhibition (%)[b]	Compound	Cell viability (%)[b]	NO inhibition (%)[b]
Lipopolysaccharide(+)	99.75 ± 0.5	0.00 ± 0.00	Indomethacin	92.22 ± 6.081	40.19 ± 8.097
5a	84.57 ± 3.10	30.07 ± 3.28	**10a**	86.64 ± 7.434	33.69 ± 6.87
5b	83.41 ± 7.18	36.26 ± 3.74	**10b**	92.47 ± 8.097	35.00 ± 8.37
5c	88.25 ± 8.466	20.19 ± 4.38	**10c**	84.1 ± 4.562	35.92 ± 6.49
5d	86.32 ± 8.968	37.59 ± 7.88	**10d**	87.34 ± 1.176	74.02 ± 5.35
5e	94.24 ± 8.186	38.83 ± 2.92	**10e**	87.65 ± 2.245	49.72 ± 6.51
5f	88.31 ± 7.326	38.06 ± 5.51	**10f**	93.2 ± 7.168	24.79 ± 8.52
5g[c]	83.57 ± 1.226	36.79 ± 0.60	**10g**[c]	77.18 ± 3.467	44.07 ± 0.76
5h	75.05 ± 8.411	55.87 ± 3.053	**10h**	91.83 ± 4.244	64,61 ± 2.43
5i	88.26 ± 6.534	30.41 ± 3.81	**10i**	89.89 ± 5.464	45,05 ± 10.43
5j	81.98 ± 7.92	26.79 ± 6.74	**10j**	89.83 ± 0.663	36.54 ± 1.53
5k	85.7 ± 2.98	26.87 ± 4.42	**10k**	94.67 ± 3.455	No detection
5l	87.67 ± 4.322	34.13 ± 4.61	**10l**	88.26 ± 7.168	11.98 ± 5.30
5m	83.13 ± 6.392	41.45 ± 4.50	**10m**	81.15 ± 4.714	15.54 ± 6.11
5n	87.88 ± 6.911	32.77 ± 4.98	**10n**	78.22 ± 7.202	14.80 ± 6.25
5o	92.04 ± 7.308	17.97 ± 5.50	**10o**	89.11 ± 7.555	25.74 ± 1.27
			10p	85.53 ± 2.204	39.03 ± 5.20

[a]100-μM treatment.
[b]Data are expressed as mean ± standard deviation ($n = 3$).
[c]50-μM treatment.

determined via cell viabilities that were above 70% in comparison to the lipopolysaccharide(+) (LPS(+); Table 2). According to the results, tested compounds were found safe at 100-μM dose level, except compounds **5g** and **10g** that were safely used at 50 μM concentration.

2.2.2 | In vitro nitric oxide (NO) inhibition

As an initial anti-inflammatory activity screening, the LPS-induced NO inhibition (%) potential of the synthesized compounds was applied with the Griess assay method. The results are summarized in Table 2, and it is observed that all tested compounds were able to cause NO inhibition except **10k**. NO inhibition results also point out that the ibuprofen derivatives (**10a–p**) were more active than the salicylic acid series (**5a–o**). Particularly, compounds **10d** and **10h** exhibited an excellent nitrite-suppressor activity, compared with indomethacin. Also, NO inhibition values of compounds **5h**, **10e**, **10g**, **and 10i** were higher than the activity of reference indomethacin. These results reveal that halogen fragments have a significant role in NO inhibition. Especially, 3-trifluoromethyl in **10d**, 2,3-dichloro in **5h**, and **10h** derivatives demonstrate the importance of halogen atom to obtain the desired response.

2.2.3 | In vitro COX-1 and COX-2 inhibition

The synthesized compounds were tested for their ability to inhibit COX-1 and COX-2 enzymes using in vitro fluorometric assay (Catalog no. 700100; Cayman Chemicals). The anti-inflammatory activity of compounds against the enzyme level was compared with the known reference drug ibuprofen, using kit-specific standards. Results are presented in Table 3 as the percentage of enzyme inhibition.

The results for COX inhibition assays indicated that the title compounds inhibited COX-1 more selectively than the COX-2 enzyme. Especially, ibuprofen-derived compound **10b**, which contains 4-fluorophenyl moiety, displayed highest activity, with 76% for COX-1 inhibition. Moreover, compounds **5a**, **5e**, **5i**, **5k**, **5n**, **10b**, **10e**, and **10h** showed more than 50% COX-1 inhibitor response.

2.2.4 | In vitro PGE_2 inhibition

On the basis of the cooperation between NO and PGE_2, the synthesized compounds that have good and potent NO production inhibitory activities were chosen and studied further for PGE_2 responses. Compounds **5i**, **5n**, and **10b** that showed the highest COX inhibition were added to this group to evaluate their

TABLE 3 COX-1 and COX-2 inhibition of compounds **5a–o** and **10a–p**

Compound	COX-1 inhibition (%)	COX-2 inhibition (%)	Compound	COX-1 inhibition (%)	COX-2 inhibition (%)
5a	**51.3 ± 1.6**	26.9 ± 1.8	**10a**	19.3 ± 1.0	10.2 ± 3.4
5b	24.1 ± 1.0	10.5 ± 1.4	**10b**	**76.4 ± 2.1**	9.9 ± 1.2
5c	24.7 ± 0.8	20.5 ± 0.5	**10c**	35.6 ± 1.1	20.7 ± 2.2
5d	32.3 ± 1.9	25.6 ± 0.7	**10d**	31.5 ± 1.4	13.9 ± 1.0
5e	**50.3 ± 2.1**	39.8 ± 0.6	**10e**	**51.7 ± 2.8**	20.2 ± 2.5
5f	47.9 ± 2.4	20.4 ± 4.1	**10f**	44.2 ± 3.6	35.3 ± 0.5
5g	14.2 ± 2.8	8.6 ± 0.8	**10g**	47 ± 0.8	39.3 ± 0.3
5h	40.4 ± 0.3	18.6 ± 1.9	**10h**	**54.7 ± 2.2**	32.4 ± 0.4
5i	**56.7 ± 0.8**	20.8 ± 1.8	**10i**	9.8 ± 1.1	6.7 ± 1.2
5j	35.2 ± 3.1	14.8 ± 1.9	**10j**	28.4 ± 2.3	23.2 ± 1.4
5k	**51.8 ± 2.6**	19.7 ± 1.2	**10k**	12.8 ± 1.5	6.8 ± 1.5
5l	17.3 ± 2.2	9 ± 2.5	**10l**	31.2 ± 0.6	10.3 ± 1.5
5m	40.8 ± 2.3	28.7 ± 3.2	**10m**	48.3 ± 0.3	20.5 ± 1.1
5n	**56 ± 4.8**	33.8 ± 3.3	**10n**	32.7 ± 1.3	24 ± 4.8
5o	41.8 ± 3.7	18.3 ± 4.2	**10o**	18.2 ± 2.8	12.5 ± 3.7
			10p	26.7 ± 2.0	20.3 ± 4.5
			Ibuprofen	**83.8 ± 1.8**	**62.7 ± 4.0**
			SC-560[a]	**90 ± 2.6**	**NI**
			DuP-697[b]	**NI**	**90 ± 2.6**

Abbreviations: COX, cyclooxygenase; NI, no inhibition.
[a]SC-560: Selective COX-1 inhibitor.
[b]DuP-697: Selective COX-2 inhibitor.

anti-inflammatory activities in detail. This target set of compounds expressed varying inhibitory activities for the LPS-induced NO and PGE2 productions. Results of these additional assays are summarized in Table 4.

It is noticeable that the target compounds are more active as inhibitors of NO production rather than PGE_2 production. The results indicated that compounds **5h**, **10d**, **10e**, **10g**, **10h**, and **10i** showed a better NO inhibitory profile than the activity of indomethacin, which is also found statistically significant. Besides, the other test compounds, **5d**, **5e**, **5f**, **5k**, **5n**, **10b**, **10j**, and **10p**, also decreased the NO production in moderate activities of LPS-induced Raw 264.7 cells. These results implied that halogen substitution is somewhat additionally beneficial for the activity. However, all of the compounds that displayed a considerable activity in NO production did not show a potent inhibitory effect on the PGE_2 production.

2.3 | Docking studies

To reveal the possible interactions of the compounds with COX enzymes, compounds that showed an anti-inflammatory profile in screening tests and possessed more than 50% inhibition in the enzyme assays were docked into COX-1 (PDB ID: 5U6X[22]) and COX-2 (PDB ID: 5IKT[23]) enzymes. Docking scores and predicted interactions of the data set are given in Table 5.

Binding to COX-1 enzyme generally occurs by an interaction with Arg120, Tyr355, and catalytic Tyr385 residues. Docking studies on COX-1 enzyme were performed by using the X-ray data of 5U6X, in which the cocrystallized ligand and its conformers made a π–π interaction with Tyr355 from isoxazole ring and a halogen bond with the Arg120 residue (docking score: −7.715, the root mean square deviation (RMSD: 0.210).[22] Similarly, the *S* enantiomer of compound **10b** (docking score: −8.695) could make two H-bonds with Arg120 in addition to the π–π interaction with Tyr355 from oxadiazole ring, which may explain its high inhibition observed by in vitro studies. The compound could also interact with Trp387 by π–π stacking from the 4-fluorophenyl group. Docking studies revealed that the *R* enantiomer of the compound **10b** could make an additional π–cation interaction with Arg120 (docking score: −8.386; Figures 1a and 2a).

Mutation studies proved that the interaction with Arg120 is not as crucial for binding to COX-2 enzyme as COX-1.[24] The conformer of cocrystallized ligand with the lowest docking score and the most stable binding pose (−10.165, RMSD: 0.738) made H-bonds with Tyr385 and Ser530 from free carboxylate group within the docking studies performed on X-ray data of 5IKT.[23] However, both enantiomers of compound **10b** (docking score (*S*): −8.006, docking score (*R*): −7.837)

TABLE 4 Cytotoxities, NO, and prostaglandin E_2 (PGE_2) production inhibition of selected compounds

Compound	IC_{50} (μM)	NO production (μM)	PGE_2 level (pg/ml)
LPS (+)		**41.17 ± 2.96**	**2,396 ± 154.8**
5d	>100	27.47 ± 3.99*	2,227 ± 14.41
5e	>100	27.33 ± 3.51*	2,156 ± 55.78*
5f	>100	27.74 ± 4.02*	2,198 ± 42.66
5h	>100	**20.75 ± 4.40****	**354.2 ± 36.54****
5k	>100	31.95 ± 2.29	1,521 ± 27.5**
5m	>100	26.13 ± 3.00**	2,392 ± 0.1
5n	>100	29.29 ± 2.05	1,659 ± 57.5**
10b	>100	30.52 ± 6.70	2,285 ± 219.5
10d	>100	**13.16 ± 4.84****	1,982 ± 38.46**
10e	>100	**23.77 ± 5.55*****	2,142 ± 13.85*
10g	68.47 ± 1.80	**23.02 ± 1.51****	2,377 ± 76.84
10h	>100	**14.51 ± 0.13****	**1,026 ± 59.68****
10i	>100	**24.02 ± 3.90*****	2,300 ± 29.76
10j	>100	28.16 ± 2.93*	2,330 ± 60.29
10p	>100	27.96 ± 4,83*	2,454 ± 31.75
Indomethacin	>100	**24.46 ± 1.80*****	**22.94 ± 4.26****

Note: One-way analysis of variance, followed by Tukey's post-hoc tests. The significant differences between groups and control (lipopolysaccharide(+), LPS) were defined with *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

lacked the interactions with Tyr385 and Ser530, which may explain their selective inhibition against COX-1 enzyme observed by in vitro studies. Instead, they could both make a π–cation interaction with Arg120 and a π–π interaction with Tyr355 from oxadiazole ring (Figures 1b and 2b).

3 | CONCLUSİON

In this study, two sets of Mannich bases from salicylic acid and ibuprofen were designed, synthesized, and evaluated for their in vitro COX-1/2 inhibitory activities and NO and PGE_2 productions. Among the compounds, **5h**, **10d**, **10e**, **10g**, **10h**, and **10j** were found to have a significant anti-inflammatory activity in NO production in comparison to the reference drug indomethacin. The in silico studies were also utilized to predict the binding interactions of these compounds with COX-1 and COX-2. The active compound set showed a significant interaction with Arg120 residue present in both enzymes. However, lack of interactions with Tyr385 and Ser530 of COX-2 was predicted as a reason of selective inhibition against COX-1 enzyme, which leads to the gastrointestinal side effects of anti-inflammatory drugs. These results indicate that although ibuprofen-derived compounds might be regarded as promising anti-inflammatory agents, further studies should be conducted for the development of safer and more active anti-inflammatory agents.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All reagents, catalysts, and solvents were purchased from commercial suppliers. Thin-layer chromatography was performed on precoated silica gel 60 F245 aluminum plates (Merck) with visualization under ultraviolet light. Melting points were determined on Mettler Toledo FP62 capillary melting point apparatus with open capillary tubes, and they were uncorrected. IR spectra were recorded using KBr pellets in PerkinElmer Spectrum One series FT-IR apparatus (Version 5.0.1.), and ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA), using tetramethylsilane as the internal reference and dimethyl sulfoxide (DMSO-d_6) as solvent; the chemical shifts were recorded in parts per million (ppm). Coupling constants were reported in Hertz. Elemental analyses were performed on LECO 932 (St. Joseph, MI) instrument.

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

4.1.2 | General procedure for the preparation of methyl esters 2 and 7

A solution of 1-g salicylic acid (**1**) (0.007 mol) or ibuprofen (**6**) (0.005 mol), 10-ml methanol, and 0.75-ml concentrated sulfuric acid was prepared. The mixture was gently refluxed at 85°C for about 75 min by mixing. At the end of the reaction, the solution mixture was cooled and extracted with hexane. The organic layer was taken and evaporated. Isolated ester compounds were checked by thin-layer chromatography with the benzene/methanol (90:10) mobile phase system.[25]

4.1.3 | General procedure for the preparation of aroylhydrazides 3 and 8

The mixture of 0.1-mol methyl esters of salicylic acid (**2**) or ibuprofen (**7**) and 0.2 mol of hydrazine hydrate was refluxed in 20 ml absolute alcohol for about 10–20 hr. The excess solvent was evaporated under reduced pressure and the concentrated solution was quenched into ice-cold water. The precipitated white solid was filtered, rewashed with water, and dried for about 2 days. The crude product was purified by recrystallization from water to produce pure hydrazide compounds. Isolated hydrazide compounds were checked by thin-layer chromatography with the benzene/methanol (40:60) mobile phase system.[18]

TABLE 5 Docking scores and predicted interactions of the chosen derivatives with COX-1 and COX-2 enzymes

Compound	Docking Score (COX-1)	Y355 (COX-1)	R120 (COX-1)	W387 (COX-1)	Docking Score (COX-2)	S530 (COX-2)	Y385 (COX-2)	R120 (COX-2)	Y355
5a	−7.688	H-bond, π–π stacking	H-bond, π–cation		−8.03			π–cation	
5d	−7.668	H-bond			−8.064				π–π stacking
5e	−7.805	π–π stacking	H-bond		−8.068				π–π stacking
5h	−7.632	π–π stacking	H-bond, π–cation		−7.199				π–π stacking
5i	−7.863	π–π stacking	H-bond		−8.178				π–π stacking
5k	−7.079		π–cation		−7.991	H-bond		π–cation	
5m	−8.003	H-bond, π–π stacking	H-bond, π–cation		−8.483		π–π stacking		
5n	−7.444	H-bond, π–π stacking	H-bond, π–cation	π-π stacking	−8.286			H-bond	
10b (*S*)	−8.695	π–π stacking	H-bond	π–π stacking	−8.006			π–cation	π–π stacking
10b (*R*)	−8.386	π–π stacking	H-bond, π–cation		−7.837			π–cation	π–π stacking
10d	−8.951		H-bond		−7.5			H-bond, π–cation	H-bond
10e	−8.658	π–π stacking	H-bond	π–π stacking	−7.985			π–cation	π–π stacking
10g	−8.627	π–π stacking	H-bond, π–cation		−7.851			π–cation	π–π stacking
10h	−8.444	π–π stacking			−6.389			H-bond, π–cation	
10i	−7.600	π–π stacking			−7.907			π–cation	π–π stacking
10m	−7.697	π–π stacking	H-bond, π–cation	π–π stacking	−7.471			π–cation	π–π stacking
10p	−8.062		π–cation		−7.531			π–cation	π–π stacking
Cocrystallized ligand (PDB ID: 5U6X)	−7.715 (RMSD: 0.210)	π–π stacking	Halogen bond						
Cocrystallized ligand (PDB ID: 5IKT)					−10.165 (RMSD: 0.738)	H-bond	H-bond		

Abbreviations: COX, cyclooxygenase; RMSD, root mean square deviation.

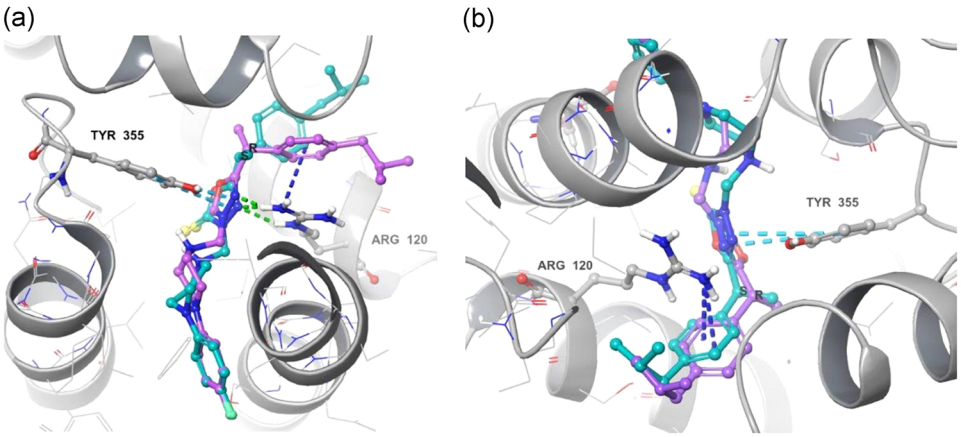


FIGURE 1 Docking pose of **10b**-*R* (purple) and **10b**-*S* (cyan). (a) COX-1: PDB ID: 5U6X; (b) COX-2: PDB ID: 5IKT

4.1.4 | General procedure for the preparation of 5-substituted-1,3,4-oxadiazole-2-(3*H*)-thiones (4, 9)

The mixture of aroyl hydrazide (**3**, **8**) (0.1 mol), KOH (0.1 mol) in absolute alcohol (50 ml), and CS_2 (0.2 mol) was taken in a round bottom flask and refluxed for about 20–25 hr until the evolution of hydrogen sulfide had ceased. The reaction mixture was cooled to room temperature and diluted with water. The product precipitated after the acidification with 10% hydrochloric acid was filtered and thoroughly crystallized with hexane.[18] Isolated 5-substituted-1,3,4-oxadiazole-2(3*H*)-thione compounds were checked by thin-layer chromatography with the benzene/methanol (90:10) mobile phase system.

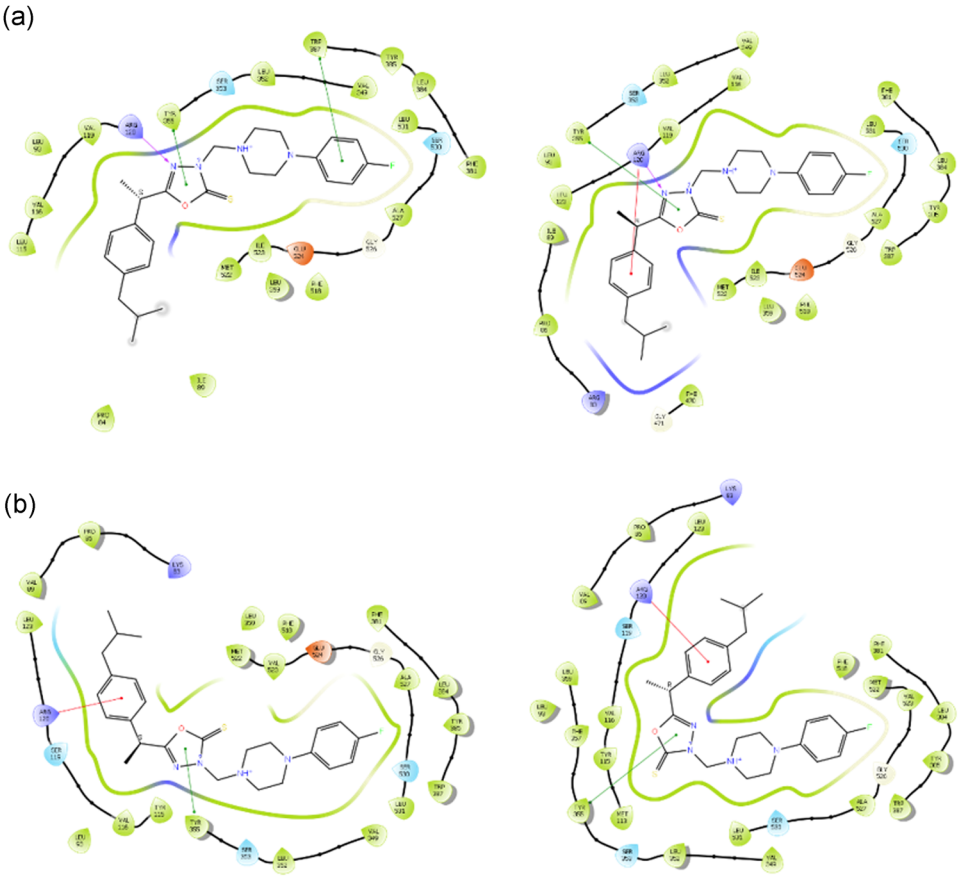


FIGURE 2 Predicted interactions of **10b**-*R* (right) and **10b**-*S* (left). (a) COX-1: PDB ID: 5U6X; (b) COX-2: PDB ID: 5IKT

4.1.5 | General procedure for the preparation of the Mannich bases (5a–o, 10a–p)

A mixture of 5-substituted-1,3,4-oxadiazole-2(3*H*)-thione (**4**, **9**) (0.01 mol), formaldehyde (0.015 mol), and piperazine derivative (0.01 mol) in 15 ml ethanol or methanol was continuously reacted at room temperature overnight. The precipitated solids in reaction media were filtered and recrystallized from ethanol or methanol to obtain the desired Mannich bases.[18]

*5-(2-Hydroxyphenyl)-3-[(4-phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5a**)*[19]

Compound **5a** was obtained as a white powder (84%). Mp: 170°C. IR ν_{max} (cm^{-1}): 3,332 (O–H), 2,886 (aromatic C–H), 1,626 (C═N), 1,598, 1,573, 1,502, 1,489 (aromatic C═C), 1,326 (C–O), 1,223 (C═S), 766 (phenyl C–H), and 746 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.90 (4H, t, J = 4.8 Hz, piperazin $H_2 + H_6$), 3.13 (4H, t, J = 4.8 Hz, piperazin $H_3 + H_5$), 5.1 (2H, s, –N–CH_2–N–), 6.76 (1H, t, J = 7.2 Hz, phenyl H_4'), 6.90 (2H, bd, J = 8 Hz, phenyl $H_2' + H_6'$), 6.95–6.99 (1H, m, phenyl H_5), 7.05 (1H, bd, J = 8.4 Hz, phenyl H_6), 7.18 (2H, t, J = 7.8 Hz, phenyl $H_3' + H_5'$), 7.42–7.46 (1H, m, phenyl H_4), 7.66 (1H, dd, J = 8 Hz, J' = 1.6 Hz, phenyl H_3), and 10.51 (1H, bs, –OH).

*5-(2-Hydroxyphenyl)-3-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5b**)*

Compound **5b** was obtained as a white powder (76%). Mp: 160°C. IR ν_{max} (cm^{-1}): 3,425 (O–H), 2,956 (aromatic C–H), 1,613 (C═N); 1,598, 1,499, 1,490 (aromatic C═C), 1,404 (C–O), 1,259 (C═S), 809 (4-fluorophenyl C–H), and 746 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.86 (4H, t, J = 5 Hz, piperazin $H_2 + H_6$), 3.06 (4H, t, J = 5 Hz, piperazin $H_3 + H_5$), 5.09 (2H, s, –N–CH_2–N–), 6.90 (1H, d, J = 4.8 Hz, phenyl H_6'), 6.92 (1H, d, J = 4.8 Hz, phenyl H_2'), 6.94–6.98 (1H, phenyl H_5), 7.00 (1H, d, J = 8.8 Hz, phenyl H_5'), 7.02 (1H, d, J = 8.8 Hz, phenyl H_3'), 7.03 (1H, d, J = 8.4 Hz, phenyl H_6), 7.40–7.44 (1H, m, phenyl H_4), 7.64 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.51 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 49.08 (piperazine $C_2 + C_6$), 49.57 (piperazine $C_3 + C_5$), 69.53 (–N–CH_2–N–), 109.00 (phenyl C_1), 115.09, 115.31 (phenyl $C_2' + C_6'$), 117.04 (phenyl C_3) 117.39, 117.47 (phenyl $C_3' + C_5'$), 119.46 (phenyl C_5), 129.09 (phenyl C_6), 133.63 (phenyl C_4), 147.87, 147.89 (phenyl C_1'), 154.90 (phenyl C_2), 156.40 (C═N), 157.25, 158.02 (phenyl C_4'–F), and 177.11 (C═S). Anal. calcd. for $C_{19}H_{19}FN_4O_2S$: C, 59.05; H, 4.96; N, 14.50; S, 8.30. Found: C, 58.56; H, 4.99; N, 14.52; S, 8.52.

*5-(2-Hydroxyphenyl)-3-({4-[4-(trifluoromethyl)phenyl]piperazin-1-yl}methyl)-1,3,4-oxadiazole-2(3H)-thione (**5c**)*

Compound **5c** was obtained as a white powder (60%). Mp: 161°C. IR ν_{max} (cm^{-1}): 3,336 (O–H), 2,837 (aromatic C–H), 1,618 (C═N), 1,598, 1,577, 1,522, 1,490 (aromatic C═C), 1,334 (C–O), 1,256 (C═S), 823 (4-trifluoromethylphenyl C–H), and 746 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm), 2.88 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.28 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 5.09 (2H, s, –N–CH_2–N–), 6.93–6.97 (1H, m, phenyl H_5), 7.03 (3H, bd, J = 8.8 Hz, phenyl $H_3' + H_5' + H_6$), 7.40–7.44 (1H, phenyl H_4), 7.46 (2H, bd, J = 8.8 Hz, phenyl $H_2' + H_6'$), 7.64 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.51 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 46.98 (piperazine $C_2 + C_6$), 49.31 (piperazine $C_3 + C_5$), 69.49 (–N–CH_2–N–), 108.99 (phenyl C_1), 114.32 (phenyl $C_2' + C_6'$), 117.04 (phenyl C_3), 117.43, 117.76, 118.07, 118.39 (phenyl C_4'–CF_3), 119.44 (phenyl $C_3' + C_5'$), 123.57 (phenyl C_6), 126.08, 126.11 (phenyl C_4'–CF_3), 129.12 (phenyl C_5), 133.65 (phenyl C_4), 153.16 (phenyl C_1'), 156.40 (phenyl C_2), 158.03 (C═N), and 177.09 (C═S). Anal. calcd. for $C_{20}H_{19}F_3N_4O_2S$: C, 55.04; H, 4.39; N, 12.84; S, 7.35. Found: C, 54.86; H, 4.47; N, 12.90; S, 7.46.

*5-(2-Hydroxyphenyl)-3-({4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}methyl)-1,3,4-oxadiazole-2(3H)-thione (**5d**)*

Compound **5d** was obtained as a white powder (30%). Mp: 173°C. IR ν_{max} (cm^{-1}): 3,348 (O–H), 2,961 (aromatic C–H), 1,627 (C═N), 1,598, 1,571, 1,491 (aromatic C═C), 1,331 (C–O), 1,250 (C═S), 849 (3-(trifluoromethyl)phenyl C–H), and 743 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.89 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.22 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 5.09 (2H, s, –N–CH_2–N–), 6.95 (1H, t, J = 7.8 Hz, phenyl H_5'), 7.03 (2H, bd, J = 8.4 Hz, phenyl $H_4' + H_6'$), 7.14 (1H, bs, phenyl H_2'), 7.18–7.23 (1H, m, phenyl H_5), 7.36 (1H, bd, J = 8 Hz, phenyl H_6), 7.40–7.45 (1H, m, phenyl H_4), 7.64 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.53 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 47.60 (piperazine $C_2 + C_6$), 49.36 (piperazine $C_3 + C_5$), 69.44 (–N–CH_2–N–), 108.93 (phenyl C_5), 111.03, 111.07 (phenyl C_2'), 114.65, 114.69 (phenyl C_4'), 117 (phenyl C_4), 118.91 (phenyl C_6'), 119.39 (phenyl C_6), 122.97, 125.68 (phenyl C_3'–CF_3), 129.11 (phenyl C_3), 129.84 (phenyl C_5'), 129.31, 129.61, 129.92, 130.22 (phenyl C_3'–CF_3), 133.64 (phenyl C_1), 151.13 (phenyl C_1'), 156.37 (phenyl C_2), 157.92 (C═N), and 177.02 (C═S). Anal. calcd. for $C_{20}H_{19}F_3N_4O_2S$: C, 55.04; H, 4.39; N, 12.84; S, 7.35. Found: C, 54.91; H, 4.29; N, 12.79; S, 7.42.

*5-(2-Hydroxyphenyl)-3-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5e**)*

Compound **5e** was obtained as a white powder (56%). Mp: 150°C. IR ν_{max} (cm^{-1}): 3,348 (O–H), 2,948 (aromatic C–H), 1,622 (C═N), 1,596, 1,569, 1,496, 1,489 (aromatic C═C), 1,323 (C–O), 1,235 (C═S), 815 (4-chlorophenyl C–H), and 742 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.87 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.12 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 5.08 (2H, s, –N–CH_2–N–), 6.91 (2H, bd, J = 9 Hz, phenyl $H_2' + H_6'$), 6.94–6.98 (1H, m, phenyl H_5), 7.03 (1H, bd, J = 8 Hz, phenyl H_6), 7.19 (2H, bd, J = 9 Hz, phenyl $H_3' + H_5'$), 7.41–7.45 (1H, m, phenyl H_4), 7.64 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.52 (1H, s, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 48.06 (piperazine $C_2 + C_6$), 49.42 (piperazine $C_3 + C_5$), 69.51 (–N–CH_2–N–), 108.99 (phenyl C_5), 117.52 (phenyl $C_2' + C_6' + C_4$), 119.44 (phenyl C_6), 122.47 (phenyl C_4'), 128.55 (phenyl $C_3' + C_5'$), 129.15 (phenyl C_3), 129.15 (phenyl C_3), 133.67 (phenyl C_1), 149.75 (phenyl C_1'), 156.41 (phenyl C_2), 157.97 (C═N), and 177.07 (C═S). Anal. calcd. for $C_{19}H_{19}ClN_4O_2S$: C, 56.64; H, 4.75; N, 13.91; S, 7.96. Found: C, 56.49; H, 4.88; N, 13.84; S, 7.98.

*5-(2-Hydroxyphenyl)-3-{[4-(2-chlorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5f**)*

Compound **5f** was obtained as a white powder (32%). Mp: 180°C. IR ν_{max} (cm^{-1}): 3,435 (O–H), 3,012 (aromatic C–H), 1,615 (C═N), 1,603, 1,560, 1,484 (aromatic C═C), 1,311 (C–O), 1,264 (C═S), 772 (2-chlorophenyl C–H s), and 743 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.93 (4H, d, J = 4.8 Hz, piperazine $H_2 + H_6$), 2.97 (4H, d, J = 4.8 Hz, piperazine $H_3 + H_5$), 5.1 (2H, s, –N–CH_2–N–), 6.98 (1H, t, J = 7.6 Hz, phenyl H_5'), 7.00–7.04 (1H, m, phenyl H_5), 7.05 (1H, bd, J = 8 Hz, phenyl H_6'), 7.14 (1H, dd, J = 7.8 Hz, J' = 1.4 Hz, phenyl H_6), 7.25–7.30 (1H, m, phenyl H_4'), 7.37 (1H, dd, J = 8 Hz, J' = 1.2 Hz, phenyl H_3'), 7.43–7.47 (1H, m, phenyl H_4), 7.68 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.54 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 49.82 (piperazine C2 + C6), 50.78 (piperazine C3 + C5), 69.69 (–N–CH2–N–), 108.98 (phenyl C5), 117.07 (phenyl C4), 119.48 (phenyl C6), 120.90 (phenyl C6′), 123.95 (phenyl C4′), 127.61 (phenyl C2′), 128.01 (phenyl C5′), 129.11 (phenyl C3), 130.27 (phenyl C3′), 133.67 (phenyl C1), 148.86 (phenyl C1′), 156.42 (phenyl C2), 157.99 (C═N), and 177.09 (C═S). Anal. calcd. for $C_{19}H_{19}ClN_4O_2S$: C, 56.64; H, 4.75; N, 13.91; S, 7.96. Found: C, 56.71; H, 4.88; N, 13.88; S, 8.05.

*5-(2-Hydroxyphenyl)-3-{[4-(3,4-dichlorophenyl)piperazin-1-yl]-methyl}-1,3,4-oxadiazole-2(3H)-thione (**5g**)*

Compound **5g** was obtained as a white powder (75%). Mp: 172°C. IR ν_{max} (cm^{-1}): 3,336 (O–H), 2,837 (aromatic C–H), 1,618 (C═N), 1,598, 1,577, 1,522, 1,490 (aromatic C═C), 1,334 (C–N), 1,256 (C═S), 823 (3,4-dichlorophenyl C–H), and 746 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.88 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.39 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 5.1 (2H, s, –N–C$\underline{H}_2$–N–); 6.92 (1H, dd, J = 9 Hz, J' = 3 Hz, phenyl H_6'), 6.95–6.99 (2H, m, phenyl $H_4 + H_5$), 7.03 (1H, bd, J = 8 Hz, phenyl H_5'); 7.13 (1H, d, J = 2.4 Hz, phenyl H_2'), 7.37 (1H, d, J = 8.8 Hz, phenyl H_6), 7.46 (1H, d, J = 9.2 Hz, phenyl H_3), and 10.61 (1H, bs, –O$\underline{H}$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 42.43 (piperazine $C_2 + C_6$), 44.85 (piperazine $C_3 + C_5$), 69.40 (–N–C$\underline{H}_2$–N–), 109.31 (phenyl C_5), 115.45 (phenyl C_4), 115.84 (phenyl C_6), 116.33 (phenyl C_6'), 116.62 (phenyl C_5'), 116.98 (phenyl C_2'), 119.49 (phenyl C_3), 120.78 (phenyl C_4'), 130.34 (phenyl C_3'), 130.56 (phenyl C_1), 131.57 (phenyl C_1'), 149.61 (phenyl C_2), 150.61 (C═N), and 155.91 (C═S). Anal. calcd. for $C_{19}H_{18}Cl_2N_4O_2S$: C, 52.18; H, 4.15; N, 12.81; S, 7.33. Found: C, 51.98; H, 4.02; N, 12.83; S, 7.39.

*5-(2-Hydroxyphenyl)-3-{[4-(2,3-dichlorophenyl)piperazin-1-yl]-methyl}-1,3,4-oxadiazole-2(3H)-thione (**5h**)*

Compound **5h** was obtained as a white powder (40%). Mp: 165°C. IR ν_{max} (cm^{-1}): 3,348 (O–H), 2,961 (aromatic C–H), 1,627 (C═N), 1,610, 1,598, 1,571, 1,491 (aromatic C═C), 1,308 (C–O), 1,265 (C═S), 849 (2,3-dichlorophenyl C–H), and 743 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.93–2.99 (8H, m, piperazine $H_2 + H_3 + H_5 + H_6$), 5.1 (2H, s, –N–CH_2–N–), 6.98 (1H, t, J = 7.4 Hz, phenyl H_5'), 7.05 (1H, bd, J = 8 Hz, phenyl H_4'), 7.12–7.16 (1H, m, phenyl H_5), 7.27–7.32 (2H, m, phenyl $H_6 + H_6'$), 7.43–7.47 (1H, m, phenyl H_4), 7.67 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.54 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 49.77 (piperazine $C_2 + C_6$), 50.87 (piperazine $C_3 + C_5$), 69.65 (–N–CH_2–N–), 108.99 (phenyl C_1), 117.07 (phenyl C_3), 119.49 (phenyl C_5), 119.67 (phenyl C_2'), 124.49 (phenyl C_6'), 126.06 (phenyl C_6), 128.40 (phenyl C_4'), 129.09 (phenyl C_5'), 132.56 (phenyl C_4), 133.65 (phenyl C_3'), 151.03 (phenyl C_1'), 156.41 (phenyl C_2), 158.06 (C═N), and 177.11 (C═S). Anal. calcd. for $C_{19}H_{18}Cl_2N_4O_2S$: C, 52.18; H, 4.15; N, 12.81; S, 7.33. Found: C, 51.75; H, 4.17; N, 13.52; S, 7.07.

*5-(2-Hydroxyphenyl)-3-[4-(4-methylphenyl)piperazin-1-yl]methyl-1,3,4-oxadiazole-2(3H)-thione (**5i**)*

Compound **5i** was obtained as a white powder (51%). Mp: 165°C. IR ν_{max} (cm^{-1}): 3,342 (O–H), 3,028 (aromatic C–H), 1,624 (C═N), 1,597, 1,514, 1,498 (aromatic C═C), 1,324 (C–O), 1,260 (C═S), 767 (4-methylphenyl C–H), and 745 (2-hydroxyphenyl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.16 (phenyl –CH_3), 2.87 (4H, d, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.05 (4H, d, J = 4.8 Hz, piperazine $H_3 + H_5$), 5.08 (2H, s, –N–CH_2–N–), 6.79 (2H, d, J = 8.6 Hz, phenyl $H_3' + H_5'$), 6.94–6.97 (1H, m, phenyl H_5), 6.98 (1H, bd, J = 8 Hz, phenyl H_6), 7.03 (2H, d, J = 8.6 Hz, phenyl $H_2' + H_6'$), 7.39–7.44 (1H, m, phenyl H_4), 7.34 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), and 10.53 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 19.94 (phenyl –CH_3), 48.71 (piperazine $C_2 + C_6$), 49.53 (piperazine $C_3 + C_5$), 69.52 (–N–CH_2–N–), 108.93 (phenyl C_5), 115.85 (phenyl $C_2' + C_6'$), 116.99 (phenyl C_4), 119.39 (phenyl C_6), 127.75 (phenyl C_4'), 129.08 (phenyl C_3), 129.23 (phenyl $C_3' + C_5'$), 133.60 (phenyl C_1), 148.84 (phenyl C_1'), 156.35 (phenyl C_2), 157.89 (C═N), and 177.01 (C═S). Anal. calcd. for $C_{20}H_{22}N_4O_2S$: C, 62.80; H, 5.80; N, 14.65; S, 8.38. Found: C, 62.87; H, 5.96; N, 14.56; S, 8.41.

*5-(2-Hydroxyphenyl)-3-{[4-(2,3-dimethylphenyl)piperazin-1-yl]-methyl}-1,3,4-oxadiazole-2(3H)-thione (**5j**)*

Compound **5j** was obtained as a white powder (66%). Mp: 133°C. IR ν_{max} (cm^{-1}): 3,364 (O–H), 2,948 (aromatic C–H), 1,630 (C═N), 1,598, 1,492 (aromatic C═C), 1,323 (C–O), 1,255 (C═S), and 746 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.09 + 2.17 (6H, s, phenyl –CH_3), 2.79–2.91 (8H, m, piperazine $H_2 + H_3 + H_5 + H_6$), 5.1 (2H, s, –N–CH_2–N–), 6.86 (1H, t, J = 8.4 Hz, phenyl H_5'), 6.99 (1H, d, J = 4.8 Hz, phenyl H_4'), 7.00–7.09 (1H, m, phenyl H_5), 7.03 (1H, d, J = 10.8 Hz, phenyl H_6'), 7.05 (1H, d, J = 4.8 Hz, phenyl H_6), 7.39–7.48 (1H, m, phenyl H_4), 7.67 (1H, dd, J = 8 Hz, J' = 1.6 Hz, phenyl H_3), and 10.58 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 13.66, 20.21 (–CH_3), 50.14 (piperazine $C_2 + C_6$), 51.72 (piperazine $C_3 + C_5$), 69.81 (methylene –CH_2–), 108.03 (phenyl C_1), 116.44 (phenyl C_3), 117.05 (phenyl C_6'), 119.49 (phenyl C_5'), 124.62 (phenyl C_v'), 125.65 (phenyl C_4), 129.00 (phenyl C_5), 130.44 (phenyl C_6), 130.59 (phenyl C_4'), 137.22 (phenyl C_3'), 151.21 (phenyl C_1'), 156.40 (phenyl C_2), 158.05 (C═N), and 177.14 (C═S). Anal. calcd. for $C_{21}H_{24}N_4O_2S$: C, 63.61; H, 6.10; N, 14.13; S, 8.09. Found: C, 62.98; H, 6.03; N, 14.37; S, 8.27.

*5-(2-Hydroxyphenyl)-3-{[4-(4-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5k**)*

Compound **5k** was obtained as a white powder (32%). Mp: 148°C. IR ν_{max} (cm^{-1}): 3,362 (O–H), 2,947 (aromatic C–H), 1,624 (C═N), 1,596,

1,512 (aromatic C═C), 1,322 (C–O), 1,249 (C═S), 835 (4-methoxyphenyl C–H), and 744 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.88 (4H, bt, J = 4.5 Hz, piperazine $H_2 + H_6$), 2.99 (4H, bt, J = 4.5 Hz, piperazine $H_3 + H_5$), 3.65 (3H, s, phenyl –OCH_3), 5.08 (2H, s, –N–CH_2–N–), 6.77 (2H, bd, J = 9 Hz, phenyl $H_2' + H_6'$), 6.85 (2H, bd, J = 9 Hz, phenyl $H_3' + H_5'$), 6.96 (1H, t, J = 7.6 Hz, phenyl H_5), 7.03 (1H, bd, J = 8 Hz, phenyl H_6), 7.40–7.45 (1H, m, phenyl H_4), 7.65 (1H, dd, J = 7.8 Hz, J' = 1.4 Hz, phenyl H_3), and 10.52 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 49.69 (piperazine $C_2 + C_3 + C_5 + C_6$), 55.12 (phenyl –OCH_3), 69.58 (–N–CH_2–N–), 108.99 (phenyl C_5), 114.17 (phenyl $C_2' + C_6'$), 117.05 (phenyl C_4), 117.66 (phenyl $C_3' + C_5'$), 119.44 (phenyl C_6), 129.15 (phenyl C_3), 133.66 (phenyl C_1), 145.34 (phenyl C_1'), 153.12 (phenyl C_4'), 156.42 (phenyl C_2), 157.94 (C═N), and 177.07 (C═S). Anal. calcd. for $C_{20}H_{22}N_4O_3S$: C, 60.28; H, 5.56; N, 14.06; S, 8.05. Found: C, 60.13; H, 5.76; N, 14.11; S, 8.17.

*5-(2-Hydroxyphenyl)-3-{[4-(4-cyanophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5l**)*

Compound **5l** was obtained as a white powder (55%). Mp: 124°C. IR ν_{max} (cm^{-1}): 3,304 (O–H), 2,943 (aromatic C–H), 2,214 (C≡N), 1,626 (C═N), 1,605, 1,566, 1,515, 1,488 (aromatic C═C), 1,329 (C–O), 1,249 (C═S), 819 (4-cyanophenyl C–H), and 741 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.86 (8H, t, J = 5 Hz, piperazine $H_2 + H_3 + H_5 + H_6$), 5.08 (2H, s, –N–CH_2–N–), 6.93–6.97 (1H, m, phenyl H_5), 6.98 (2H, bd, J = 9.2 Hz, phenyl $H_2' + H_6'$), 7.02 (1H, bd, J = 8 Hz, phenyl H_6), 7.39–7.44 (1H, m, phenyl H_4), (2H, m, phenyl $H_3' + H_5'$), 7.63 (1H, dd, J = 8 J' = 1.6 Hz, phenyl H_3), and 10.52 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 46.26 (piperazine $C_2 + C_6$), 49.16 (piperazine $C_3 + C_5$), 69.38 (–N–CH_2–N–), 98.23 (phenyl C_4'), 108.91 (phenyl C_5), 114.07 (phenyl $C_2' + C_6'$), 116.98 (phenyl C_4), 119.36 (phenyl C_6), 119.87 (C≡N), 129.10 (phenyl C_3), 133.20 (phenyl $C_3' + C_5'$), 133.62 (phenyl C_1), 152.98 (phenyl C_1'), 156.98 (phenyl C_2), 157.89 (C═N), and 176.99 (C═S). Anal. calcd. for $C_{20}H_{19}N_5O_2S$: C, 61.05; H, 4.87; N, 17.80; S, 8.15. Found: C, 60.47; H, 4.88; N, 17.66; S, 8.25.

*5-(2-Hydroxyphenyl)-3-{[4-(2-cyanophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5m**)*

Compound **5m** was obtained as a white powder (56%). Mp: 120°C. IR ν_{max} (cm^{-1}): 3,336 (O–H), 2,964 (aromatic C–H), 2,220 (C≡N), 1,625 (C═N), 1,595, 1,573, 1,487 (aromatic C═C), 1,322 (C-O), 1,250 (C═S), 764 (2-cyanophenyl C–H), and 741 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.94 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.14 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 5.10 (2H, s, –N–CH_2–N–), 6.96 (1H, t, J = 8 Hz, phenyl H_5'), 7.04 (1H, t, J = 8 Hz, phenyl H_6'), 7.08 (1H, bd, J = 7.6 Hz, phenyl H_6), 7.14 (1H, bd, J = 8 Hz, phenyl H_3'), 7.41–7.45 (1H, m, phenyl H_4'), 7.55–7.60 (1H, m, phenyl H_5), 7.64–7.68 (2H, m, phenyl $H_3 + H_4$), and 10.52 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 49.67 (piperazine $C_2 + C_6$), 51.11 (piperazine $C_3 + C_5$), 69.55 (–N–CH_2–N–), 104.78 (phenyl C_2'), 108.98 (phenyl C_1), 117.08 (phenyl C_3), 118.14 (phenyl C_6'), 119.16 (phenyl C_5), 119.47 (phenyl C_4'), 122.10 (phenyl C_6), 129.07 (phenyl C_5'), 133.67 (phenyl C_4), 134.19 (phenyl $C_3' +$ C≡N), 134.31 (phenyl C_1'), 155.13 (phenyl C_2), 156.43 (C═N), and 177.10 (C═S). Anal. calcd. for $C_{20}H_{19}N_5O_2S$: C, 61.05; H, 4.87; N, 17.80; S, 8.15. Found: C, 60.42; H, 4.87; N, 17.65; S, 8.27.

*5-(2-Hydroxyphenyl)-3-{[4-(2-pyridyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2-(3H)-thione (**5n**)*

Compound **5n** was obtained as a white powder (59%). Mp: 148°C. IR ν_{max} (cm^{-1}): 3,435 (O–H), 3,012 (aromatic C–H), 1,615 (C═N), 1,603, 1,560, 1,484 (aromatic C═C), 1,311 (C–O), 1,264 (C═S), 772 (2-pyridyl C–H), and 743 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 22.48 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.48 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 5.08 (2H, s, –N–CH_2–N–), 6.57–6.60 (1H, m, pyridyl H_5), 6.79 (1H, t, J = 8.8 Hz, pyridyl H_6), 6.93–6.79 (1H, m, phenyl H_5), 7.01 (1H, d, J = 8 Hz, phenyl H_6), 7.40–7.44 (1H, m, phenyl H_4), 7.46–7.50 (1H, m, pyridyl H_4), 7.63 (1H, dd, J = 7.8 Hz, J' = 1.8 Hz, phenyl H_3), 8.06 (1H, dd, J = 4.6 Hz J' = 1.8 Hz, pyridyl H_3), and 10.51 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 44.64 (piperazine $C_2 + C_6$), 49.38 (piperazine $C_3 + C_5$), 69.64 (–N–CH_2–N–), 107.06 (pyridyl C_5), 108.92 (pyridyl C_4), 112.92 (phenyl C_5), 117.01 (phenyl C_4), 119.38 (phenyl C_6), 129.11 (pyridyl C_6), 133.61 (phenyl C_3), 137.38 (phenyl C_1), 147.41 (pyridyl C_3), 156.36 (phenyl C_2), 157.84 (pyridyl C_1), 158.77 (C═N), and 176.98 (C═S). Anal. calcd. for $C_{18}H_{19}N_5O_2S$: C, 58.52; H, 5.18; N, 18.96; S, 8.68. Found: C, 58.80; H, 5.34; N, 18.86; S, 8.72.

*5-(2-Hydroxyphenyl)-3-{[4-(2-pyrimidinyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**5o**)*

Compound **5o** was obtained as a white powder (60%). Mp: 149°C. IR ν_{max} (cm^{-1}): 3,336 (O–H), 3,021 (aromatic C–H), 1,624 (C═N), 1,585, 1,547, 1,489 (aromatic C═C), 1,324 (C–O), 1,262 (C═S), 798 (2-pyrimidinyl C–H), and 742 (2-hydroxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.80 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.74 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 5.09 (2H, s, –N–CH_2–N–), 6.59 (1H, t, J = 4.8 Hz, pyrimidine H_4), 6.95 (1H, m, phenyl H_5), 7.02 (1H, bd, J = 7.6 Hz, phenyl H_6), 7.40–7.44 (1H, m, phenyl H_4), 7.63 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3), 8.32 (2H, d, J = 4.8 Hz, pyrimidine $H_3 + H_5$), and 10.48 (1H, bs, –OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 43.13 (piperazine $C_2 + C_6$), 49.44 (piperazine $C_3 + C_5$), 69.73 (–N–CH_2–N–), 108.99 (phenyl C_5), 110.08 (phenyl C_4), 117.04 (pyrimidine C_4), 119.43 (phenyl C_6), 129.13 (phenyl C_3), 133.62 (phenyl C_1), 156.38 (phenyl C_2), 157.86 (pyrimidine $C_3 + C_5$, C═N), 161.04 (pyrimidine C_1), and 177.01 (C═S). Anal. calcd. for $C_{17}H_{18}N_6O_2S$: C, 55.12; H, 4.90; N, 22.69; S, 8.66. Found: C, 54.65; H, 4.93; N, 22.68; S, 8.84.

*5-[1-(4-Isobutylphenyl)ethyl]-3-[(4-phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**10a**)*

Compound **10a** was obtained as a white powder (72%). Mp: 105°C. IR ν_{max} (cm^{-1}): 2,952 (aromatic C–H), 2,831 (aliphatic C–H), 1,620 (C═N), 1,599, 1,506 (aromatic C═C), 1,441 (C–N), 1,325 (C–O), 1,258 (C═S), 849, and 756 (phenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.56 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.78–1.81 (1H, m, isobutyl –CH–), 2.41 (2H, d, J = 7.6 Hz, isobutyl –CH_2–), 2.84 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.13 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.33 (1H, q, J = 6.8 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–),

6.77 (1H, t, J = 7.2 Hz, phenyl $H_{4'}$), 6.91 (2H, d, J = 7.8 Hz, phenyl $H_3 + H_5$), 7.13 (2H, d, J = 7.8 Hz, phenyl $H_2 + H_6$), and 7.17–7.21 (4H, m, phenyl $H_{2'} + H_{3'} + H_{5'} + H_{6'}$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 22.13 + 22.14 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.09 (isobutyl –CH_2–), 44.13 (–CH–CH_3), 48.19 (piperazine $C_2 + C_6$), 49.52 (piperazine $C_3 + C_5$), 69.43 (–N–CH_2–N–), 115.58 (phenyl $C_{2'} + C_{6'}$), 118.94 (phenyl $C_{4'}$), 126.99 (phenyl $C_3 + C_5$), 128.87 (phenyl $C_{3'} + C_{5'}$), 129.44 (phenyl $C_2 + C_6$), 136.77 (phenyl C_4), 140.51 (phenyl C_1), 157.88 (phenyl $C_{1'}$), 164.10 (C=N), and 177.76 (C=S). Anal. calcd. for $C_{25}H_{32}N_4OS$: C, 68.77; H, 7.39; N, 12.83; S, 7.34. Found: C, 68.73; H, 7.59; N, 12.88; S, 7.43.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10b**)*[15]

Compound **10b** was obtained as a white powder (69%). Mp: 121°C. IR ν_{max} (cm^{-1}): 2,950 (aromatic C–H), 2,833 (aliphatic C–H), 1,614 (C=N), 1,508 (aromatic C=C), 1,447 (C–N), 1,330 (C–O), 1,254 (C=S), 826 (1,4-dialkylphenyl C–H), and 780 (4-fluorophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.55 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.77–1.81 (1H, m, isobutyl –CH–), 2.41 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.83 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.07 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.33 (1H, q, J = 6.4 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 6.93 (2H, dd, J = 9.2 Hz, J' = 4.6 Hz, phenyl $H_{2'} + H_{6'}$), 7.03 (2H, t, J = 8.8 Hz, phenyl $H_{3'} + H_{5'}$), 7.13 (2H, bd, J = 8 Hz, phenyl $H_3 + H_5$), and 7.19 (2H, bd, J = 8 Hz, phenyl $H_2 + H_6$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.48 (isobutyl –CH–), 22.11 + 22.13 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.09 (isobutyl –CH_2–), 44.13 (–CH–CH_3), 48.94 (piperazine $C_2 + C_6$), 49.48 (piperazine $C_3 + C_5$), 69.39 (–N–CH_2–N–), 115.09 (phenyl $C_{6'}$), 115.31 (phenyl $C_{2'}$), 117.29 (phenyl $C_{5'}$), 117.36 (phenyl $C_{3'}$), 126.99 (phenyl $C_3 + C_5$), 129.43 (phenyl $C_2 + C_6$), 136.75 (phenyl C_4), 140.51 (phenyl C_1), 147.74 + 147.76 (phenyl $C_{1'}$), 154.86 + 157.20 (phenyl $C_{4'}$–F), 164.07 (C=N), and 177.75 (C=S).

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10c**)*

Compound **10c** was obtained as a white powder (40%). Mp: 108°C. IR ν_{max} (cm^{-1}): 2,950 (aromatic C–H), 2,844 (aliphatic C–H), 1,603 (C=N), 1,578, 1,500 (aromatic C=C), 1,443 (C–N), 1,352 (C–O), 1,242 (C=S), 852 (1,4-dialkylphenyl C–H), and 751 (2-fluorophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.55 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.77–1.81 (1H, m, isobutyl –CH–), 2.41 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.83 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.07 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.33 (1H, q, J = 6.4 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 6.93 (2H, dd, J = 9.2 Hz, J' = 4.6 Hz, phenyl $H_{2'} + H_{6'}$), 7.03 (2H, t, J = 8.8 Hz, phenyl $H_{3'} + H_{5'}$), 7.13 (2H, bd, J = 8 Hz, phenyl $H_3 + H_5$), and 7.19 (2H, bd, J = 8 Hz, phenyl $H_2 + H_6$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.48 (isobutyl –CH–), 0.85 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.57 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.77–1.84 (1H, m, isobutyl –CH–), 2.42 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.86 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.01 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 4.35 (1H, q, J = 7 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 6.94–7.00 (1H, m, phenyl $H_{6'}$), 7.03 (1H, bd, J = 9.2 Hz, phenyl $H_{3'}$), 7.08–7.12 (2H, m, phenyl $H_{4'} + H_{5'}$), 7.15 (2H, bd, J = 8.2 Hz, phenyl $H_3 + H_5$), and 7.22 (2H, bd, J = 8.2 Hz, phenyl $H_2 + H_6$). Anal. calcd. for $C_{25}H_{31}FN_4OS$: C, 66.05; H, 6.87; N, 12.32; S, 7.05. Found: C, 66.07; H, 7.12; N, 12.35; S, 7.15.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(3-(trifluoromethyl)phenyl]piperazin-1-yl}-methyl}-1,3,4-oxadiazole-2(3H)-thione (**10d**)*

Compound **10d** was obtained as a white powder (56%). Mp: 77°C. IR ν_{max} (cm^{-1}): 2,954 (aromatic C–H), 2,886 (aliphatic C–H), 1,610 (C=N), 1,512, 1,497 (aromatic C=C), 1,447 (C–N), 1,329 (C–O), 1,242 (C=S), 1,169 (C–F), 851 (1,4-dialkylphenyl C–H), and 781 (3-trifluoromethylphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.55 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.77–1.81 (1H, m, isobutyl –CH–), 2.40 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.84 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.23 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 4.33 (1H, q, J = 7 Hz, –CH–CH_3), 5.01 (2H, s, –N–CH_2–N–), 7.05 (1H, d, J = 7.6 Hz, phenyl $H_{6'}$), 7.12 (2H, d, J = 8 Hz, phenyl $H_3 + H_5$), 7.15 (1H, bs, phenyl $H_{2'}$), 7.19 (2H, d, J = 8 Hz, phenyl $H_2 + H_6$), 7.18–7.22 (1H, m, phenyl $H_{4'}$), and 7.40 (1H, t, J = 8 Hz, phenyl $H_{5'}$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.49 (isobutyl –CH–), 22.13 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.09 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 49.83 (piperazine $C_2 + C_6$), 50.72 (piperazine $C_3 + C_5$), 69.55 (–N–CH_2–N–), 120.94 (phenyl $C_{6'}$), 122.97 + 125.68 (–CF_3), 123.96 (phenyl $C_{4'}$), 126.99 (phenyl $C_3 + C_5$), 127.58 (phenyl $C_{2'}$), 128.02 (phenyl $C_{5'}$), 129.43 (phenyl $C_2 + C_6$), 130.27 (phenyl $C_{3'}$–CF_3), 136.82 (phenyl C_4), 140.52 (phenyl C_1), 148.83 (phenyl $C_{1'}$), 164.12 (C=N), and 177.76 (C=S). Anal. calcd. for $C_{26}H_{31}F_3N_4OS$: C, 61.88; H, 6.19; N, 11.10; S, 6.35. Found: C, 61.64; H, 6.49; N, 11.14; S, 6.39.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10e**)*[18]

Compound **10e** was obtained as a white powder (54%). Mp: 99°C. IR ν_{max} (cm^{-1}): 2,949 (aromatic C–H), 2,827 (aliphatic C–H), 1,617 (C=N), 1,596, 1,496 (aromatic C=C), 1,444 (C–N), 1,329 (C–O), 1,242 (C=S), 812 (1,4-dialkylphenyl C–H), and 790 (4-chlorophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.55 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.77–1.81 (1H, m, isobutyl –CH–), 2.40 (2H, d, J = 6.8 Hz, isobutyl –CH_2–), 2.87 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.13 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.32 (1H, q, J = 7.2 Hz, –CH–CH_3), 4.99 (2H, s, –N–CH_2–N–), 6.92 (2H, bd, J = 9.4 Hz, phenyl $H_{2'} + H_{6'}$), 7.12 (2H, bd, J = 8.6 Hz, phenyl $H_3 + H_5$), 7.18 (2H, bd, J = 8.6 Hz, phenyl $H_2 + H_6$), and 7.21 (2H, bd, J = 9.4 Hz, phenyl $H_{3'} + H_{5'}$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.48 (isobutyl –CH–), 22.11 + 22.13 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.07 (isobutyl –CH_2–), 44.13 (–CH–CH_3), 47.95 (piperazine $C_2 + C_6$), 49.27 (piperazine $C_3 + C_5$), 69.36 (–N–CH_2–N–), 116.97 (phenyl $C_{2'} + C_{6'}$), 122.36 (phenyl $C_{4'}$), 126.94 (phenyl $C_3 + C_5$), 128.56 (phenyl $C_{3'} + C_{5'}$), 129.43 (phenyl $C_2 + C_6$), 136.75 (phenyl C_4), 140.51 (phenyl C_1), 149.59 (phenyl $C_{1'}$), 164.07 (C=N), and 177.73 (C=S).

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2-chlorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10f**)*

Compound **10f** was obtained as a white powder (39%). Mp: 94°C. IR ν_{max} (cm^{-1}): 3,053 (aromatic C–H), 2,819 (aliphatic C–H), 1,618 (C=N), 1,586,

1,479 (aromatic C═C), 1,443 (C–N), 1,330 (C–O), 1,250 (C═S), 845 (1,4-dialkylphenyl C–H), and 741 (2-chlorophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.85 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.58 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.79–1.83 (1H, m, isobutyl –CH–), 2.42 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.87 (4H, d, J = 4.4 Hz, piperazine $H_2 + H_6$), 2.96 (4H, d, J = 4.4 Hz, piperazine $H_3 + H_5$), 4.37 (1H, q, J = 7.2 Hz, –CH–CH_3), 5.01 (2H, s, –N–CH_2–N–), 7.02–7.06 (1H, m, phenyl H_5'), 7.15 (1H, dd, J = 8.2 Hz, J' = 1.8 Hz, phenyl H_6'), 7.16 (2H, bd, J = 8 Hz, phenyl $H_3 + H_5$), 7.23 (2H, bd, J = 8 Hz, phenyl $H_2 + H_6$), 7.27–7.31 (1H, m, phenyl H_4'), and 7.40 (2H, dd, J = 8.2 Hz, J' = 1.4 Hz, phenyl H_3'). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.50 (isobutyl –CH–), 22.13 (isobutyl –CH_3), 29.51 (–CH–CH_3), 36.09 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 49.83 (piperazine $C_2 + C_6$), 50.72 (piperazine $C_3 + C_5$), 69.55 (–N–CH_2–N–), 120.93 (phenyl C_4'), 123.96 (phenyl C_3'), 126.99 (phenyl $C_3 + C_5$), 127.58 (phenyl C_6'), 128.03 (phenyl C_5'), 129.43 (phenyl $C_2 + C_6$), 130.28 (phenyl C_1), 136.83 (phenyl C_4), 140.52 (phenyl C_1'), 148.83 (C_2'), 164.13 (C═N), and 177.78 (C═S). Anal. calcd. for $C_{25}H_{31}ClN_4OS$: C, 63.74; H, 6.63; N, 11.89; S, 6.81. Found: C, 63.05; H, 6.83; N, 12.12; S, 6.97.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(3,4-dichlorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10g**)*

Compound **10g** was obtained as a white powder (63%). Mp: 116°C. IR ν_{max} (cm^{-1}): 2,946 (aliphatic C–H), 2,846 (aliphatic C–H), 1,619 (C═N), 1,593, 1,551, 1,512, 1,486 (aromatic C═C), 1,442 (C–N), 1,330 (C–O), 1,258 (C═S), 835 (1,4-dialkylphenyl C–H), and 802 (3,4-dichlorophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.54 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.77–1.81 (1H, m, isobutyl –CH–), 2.40 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.78 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.19 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 4.32 (1H, q, J = 7.2 Hz, –CH–CH_3), 4.99 (2H, s, –N–CH_2–N–), 6.91 (1H, dd, J = 9.2 Hz, J' = 2.8 Hz, phenyl H_6'), 7.11 (1H, d, J = 3.2 Hz, phenyl H_2'), 7.12 (2H, d, J = 8 Hz, phenyl $H_3 + H_5$), 7.17 (2H, d, J = 8 Hz, phenyl $H_2 + H_6$), and 7.40 (1H, d, J = 9.2 Hz, phenyl H_5'). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.47 (isobutyl –CH–), 22.11 + 22.12 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.06 (isobutyl –CH_2–), 44.13 (–CH–CH_3), 47.44 (piperazine $C_2 + C_6$), 49.10 (piperazine $C_3 + C_5$), 69.30 (–N–CH_2–N–), 115.39 (phenyl C_6'), 116.30 (phenyl C_5'), 119.54 (phenyl C_2'), 126.94 (phenyl $C_3 + C_5$), 129.41 (phenyl $C_2 + C_6$), 130.40 (phenyl C_4'), 131.45 (phenyl C_3'), 136.74 (phenyl C_4), 140.50 (phenyl C_1), 150.45 (phenyl C_1'), 164.07 (C═N), and 177.71 (C═S). Anal. calcd. for $C_{25}H_{30}Cl_2N_4OS$: C, 59.40; H, 5.98; N, 11.08; S, 6.34. Found: C, 58.78; H, 6.12; N, 11.15; S, 6.40.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2,3-dichlorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10h**)*

Compound **10h** was obtained as a white powder (52%). Mp: 99°C. IR ν_{max} (cm^{-1}): 2,946 (aromatic C–H), 2,845 (aliphatic C–H), 1,619 (C═N), 1,593, 1,552, 1,511, 1,486 (aromatic C═C), 1,442 (C–N), 1,326 (C–O), 1,258 (C═S), 835 (1,4-dialkylphenyl C–H), and 802 (2,3-dichlorophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.57 (3H, d, J = 7.6 Hz, –CH–CH_3), 1.77–1.83 (1H, m, isobutyl –CH–), 2.41 (2H, d, J = 6.8 Hz, isobutyl –CH_2–), 2.87 (4H, d, J = 4.2 Hz, piperazine $H_2 + H_6$), 2.97 (4H, d, J = 4.2 Hz, piperazine $H_3 + H_5$), 4.36 (1H, q, J = 6.8 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 7.14 (1H, t, J = 8 Hz, phenyl H_5'), 7.15 (2H, bd, J = 7.4 Hz, phenyl $H_3 + H_5$), 7.22 (2H, bd, J = 7.4 Hz, phenyl $H_2 + H_6$), 7.28–7.31 (2H, m, phenyl $H_4' + H_6'$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.48 (isobutyl –CH–), 22.12 + 22.13 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.08 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 49.77 (piperazine $C_2 + C_6$), 50.79 (piperazine $C_3 + C_5$), 69.51 (–N–CH_2–N–), 119.68 (phenyl C_6'), 124.47 (phenyl C_4'), 126.02 (phenyl C_2'), 126.99 (phenyl $C_3 + C_5$), 128.41 (phenyl C_5'), 129.43 (phenyl $C_2 + C_6$), 132.57 (phenyl C_3'), 136.81 (phenyl C_4), 140.52 (phenyl C_1), 150.98 (phenyl C_1'), 164.14 (C═N), 177.76 (C═S). Anal. calcd. for $C_{25}H_{30}Cl_2N_4OS$: C, 59.40; H, 5.98; N, 11.08; S, 6.34. Found: C, 58.70; H, 6.11; N, 11.09; S, 6.36.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(4-methylphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10i**)*

Compound **10i** was obtained as a white powder (70%). Mp: 99°C. IR ν_{max} (cm^{-1}): 2,948 (aromatic C–H), 2,824 (aliphatic C–H), 1,620 (C═N), 1,574, 1,519 (aromatic C═C), 1,441 (C–N), 1,326 (C–O), 1,245 (C═S), 848 (1,4-dialkylphenyl C–H), and 807 (4-methylphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.55 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.77–1.81 (1H, m, isobutyl –CH–), 2.18 (3H, s, phenyl CH_3), 2.40 (2H, d, J = 7.6 Hz, isobutyl –CH_2–), 2.83 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.06 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.33 (1H, q, J = 7.2 Hz, –CH–CH_3), 4.99 (2H, s, –N–CH_2–N–), 6.81 (2H, d, J = 8.8 Hz, phenyl $H_3' + H_5'$), 7.01 (2H, d, J = 8.8 Hz, phenyl $H_2' + H_6'$), 7.13 (2H, d, J = 8.2 Hz, phenyl $H_3 + H_5$), and 7.19 (2H, d, J = 8.2 Hz, phenyl $H_2 + H_6$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.49 (isobutyl –CH–), 20.01 (phenyl –CH_3), 22.13 + 22.14 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.09 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 48.65 (piperazine $C_2 + C_6$), 49.52 (piperazine $C_3 + C_5$), 69.44 (–N–CH_2–N–), 115.85 (phenyl $C_2' + C_6'$), 126.99 (phenyl C_4'), 127.73 (phenyl $C_3 + C_5$), 129.31 (phenyl $C_3' + C_{5v}$), 129.44 (phenyl $C_2 + C_6$), 136.78 (phenyl C_4), 140.51 (phenyl C_1), 148.79 (phenyl C_1'), 164.07 (C═N), and 177.75 (C═S). Anal. calcd. for $C_{26}H_{34}N_4OS$: C, 69.30; H, 7.60; N, 12.43; S, 7.12. Found: C, 69.08; H, 7.67; N, 12.52; S, 7.21.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2,3-xylylphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10j**)*

Compound **10j** was obtained as a white powder (45%). Mp: 93°C. IR ν_{max} (cm^{-1}): 2,950 (aromatic C–H), 2,845 (aliphatic C–H), 1,618 (C═N), 1,581, 1,511, 1,470 (aromatic C═C), 1,454 (C–N), 1,326 (C–O), 1,239 (C═S), 842 (1,4-dialkylphenyl C–H), and 776 (2,3-dimethylphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.57 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.78–1.82 (1H, m, isobutyl –CH–), 2.11 + 2.19 (6H, s, phenyl –CH_3), 2.42 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.77 (4H, bd, J = 3.6 Hz, piperazine $H_2 + H_6$), 2.85 (4H, bs, piperazine $H_3 + H_5$), 4.36 (1H, q, J = 7.2 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 6.87 (2H, bd, J = 8 Hz, phenyl H_4'+ H_6'), 7.02 (2H, t, J = 7.8 Hz, phenyl H_5'), 7.15 (2H, d, J = 8 Hz, phenyl $H_3 + H_5$), and 7.22 (2H, d, J = 8 Hz, phenyl $H_2 + H_6$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 13.57 (phenyl C_4'–CH_3), 18.50 (isobutyl –CH–), 20.01 (phenyl C_3'–CH_3), 22.12 + 22.13 (isobutyl –CH_3),

29.52 (–CH–CH_3), 36.08 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 50.15 (piperazine $C_2 + C_6$), 51.65 (piperazine $C_3 + C_5$), 60.61 (–N–CH_2–N–), 116.46 (phenyl C_6'), 124.62 (phenyl C_4'), 125.66 (phenyl C_2'), 126.98 (phenyl $C_3 + C_5$), 129.43 (phenyl $C_2 + C_6$), 130.40 (phenyl C_5'), 136.85 (phenyl C_4), 137.22 (phenyl C_3'), 140.53 (phenyl C_1), 148.79 (phenyl C_1'), 164.07 (C=N), and 177.75 (C=S). Anal. calcd. for $C_{27}H_{36}N_4OS$: C, 69.79, H, 7.81, N, 12.06, S, 6.90. Found: C, 69.01, H, 7.61, N, 12.09, S, 6.85.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(4-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10k**)*[15]

Compound **10k** was obtained as a white powder (47%). Mp: 97°C. IR ν_{max} (cm^{-1}): 2,950 (aromatic C–H), 2,845 (aliphatic C–H), 1,618 (C=N), 1,581, 1,511, 1,470 (aromatic C=C), 1,454 (C–N), 1,326 (C–O), 1,239 (C=S), 842 (1,4-dialkylphenyl C–H), and 776 (2,3-dimethylphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.82 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.54 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.77–1.80 (1H, m, isobutyl –CH–), 2.40 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.82 (4H, t, J = 4.6 Hz, piperazine $H_2 + H_6$), 2.99 (4H, t, J = 4.6 Hz, piperazine $H_3 + H_5$), 3.65 (3H, s, phenyl –OCH_3), 4.32 (1H, q, J = 6.8 Hz, –CH–CH_3), 4.98 (2H, s, –N–CH_2–N–), 6.78 (2H, bd, J = 9 Hz, phenyl $H_2' + H_6'$), 6.85 (2H, bd, J = 9 Hz, phenyl $H_3' + H_5'$), 7.13 (2H, bd, J = 8.4 Hz, phenyl $H_3 + H_5$), and 7.19 (2H, d, J = 8.4 Hz, phenyl $H_2 + H_6$).

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(3-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10l**)*

Compound **10l** was obtained as a white powder (45%). Mp: 93°C. IR ν_{max} (cm^{-1}): 2,950 (aromatic C–H), 2,845 (aliphatic C–), 1,618 (C=N), 1,581, 1,511, 1,470 (aromatic C=C), 1,454 (C–N), 1,326 (C–O), 1,239 (C=S), 842 (1,4-dialkylphenyl C–H), and 776 (2,3-dimethylphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.4 Hz, isobutyl CH_3), 1.56 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.77–1.84 (1H, m, isobutyl –CH–), 2.41 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.86 (4H, t, J = 5 Hz, piperazine $H_2 + H_6$), 3.13 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.34 (1H, q, J = 7.2 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 6.36 (1H, dd, J = 8.2 Hz, J' = 2.2 Hz, phenyl H_6'), 6.44 (1H, t, J = 2.2 Hz, phenyl H_2'), 6.51 (1H, dd, J = 8.2 Hz, J' = 2.2 Hz, phenyl H_4'), 7.09 (1H, t, J = 8.4 Hz, phenyl H_5'), 7.14 (2H, d, J = 8.2 Hz, phenyl $H_3 + H_5$), and 7.20 (2H, d, J = 8.2 Hz, phenyl $H_2 + H_6$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.49 (–CH–CH_3), 22.13 + 22.14 (isobutyl –CH_3), 29.50 (isobutyl –CH–), 36.09 (isobutyl –CH_2–), 44.13 (–CH–CH_3), 48.13 (piperazine $C_2 + C_6$), 49.49 (piperazine $C_3 + C_5$), 54.82 (phenyl C_3'–OCH_3), 69.42 (–N–CH_2–N–), 101.69 (phenyl C_2'), 104.26 (phenyl C_6'), 108.18 (phenyl C_4'), 126.98 (phenyl $C_2 + C_6$), 129.44 (phenyl $C_3 + C_5$), 129.56 (phenyl C_5'), 136.76 (phenyl C_4), 140.50 (phenyl C_1), 152.23 (phenyl C_1'), 160.14 (phenyl C_3'), 164.10 (C=N), and 177.76 (C=S). Anal. calcd. for $C_{26}H_{34}N_4O_2S$: C, 66.92, H, 7.34, N, 12.01, S, 6.87. Found: C, 65.91, H, 7.57, N, 12.43, S, 7.01.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10m**)*

Compound **10m** was obtained as a white powder (41%). Mp: 98°C. IR ν_{max} (cm^{-1}): 3,049 (aromatic C–H), 2,909 (aliphatic C–H), 1,614 (C=N), 1,594, 1,498 (aromatic C=C), 1,444 (C–N), 1,324 (C–O), 1,242 (C=S), 850 (1,4-dialkylphenyl C–H), and 737 (2-methoxyphenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.85 (6H, d, J = 6.8 Hz, isobutyl CH_3), 1.58 (3H, d, J = 7.6 Hz, –CH–CH_3), 1.76–1.86 (1H, m, isobutyl –CH–), 2.42 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.85 (4H, bs, piperazine $H_2 + H_6$), 2.95 (4H, bs, piperazine $H_3 + H_5$), 3.76 (3H, s, phenyl C_2'–OCH_3), 4.37 (1H, q, J = 7 Hz, –CH–CH_3), 4.99 (2H, s, –N–CH_2–N–), 6.86–6.95 (4H, m, phenyl $H_3' + H_4' + H_5' + H_6'$), 7.16 (2H, bd, J = 8 Hz, phenyl $H_3 + H_5$), and 7.23 (2H, bd, J = 8 Hz, phenyl $H_2 + H_6$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.49 (–CH–CH_3), 22.12 + 22.13 (isobutyl –CH_3), 29.50 (isobutyl –CH–), 36.09 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 49.86 (piperazine $C_2 + C_6$), 49.93 (piperazine $C_3 + C_5$), 55.21 (phenyl C_2'–OCH_3), 69.66 (–N–CH_2–N–), 111.78 (phenyl C_6'), 118.02 (phenyl C_3'), 120.75 (phenyl C_4'), 122.51 (phenyl C_5'), 127.03 (phenyl $C_3 + C_5$), 129.43 (phenyl $C_2 + C_6$), 136.79 (phenyl C_4), 140.52 (phenyl C_1), 140.99 (phenyl C_1'), 151.88 (phenyl C_2'), 164.11 (C=N), and 177.75 (C=S). Anal. calcd. for $C_{26}H_{34}N_4O_2S$: C, 66.92, H, 7.34, N, 12.01, S, 6.87. Found: C, 65.99, H, 7.61, N, 11.92, S, 7.01.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(4-cyanophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10n**)*

Compound **10n** was obtained as a white powder (50%). Mp: 50°C. IR ν_{max} (cm^{-1}): 2,946 (aromatic C–H), 2,840 (aliphatic C–H), 2,211 (C≡N), 1,604 (C=N), 1,513 (aromatic C=C), 1,445 (C–N), 1,329 (C–O), 1,248 (C=S), 818 (1,4-dialkylphenyl C–H), and 789 (4-cyanophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.53 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.76–1.80 (1H, m, isobutyl –CH–), 2.39 (2H, d, J = 7.2 Hz, isobutyl –CH_2–), 2.80 (4H, bs, piperazine $H_2 + H_6$), 3.53 (4H, t, J = 5 Hz, piperazine $H_3 + H_5$), 4.31 (3H, q, J = 7.2 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 7.01 (2H, bd, J = 8.8 Hz phenyl $H_2' + H_6'$), 7.09 (2H, bd, J = 8 Hz phenyl $H_3 + H_5$), 7.16 (2H, bd, J = 8 Hz, phenyl $H_2 + H_6$), and 7.56 (2H, bd, J = 8.8 Hz, phenyl $H_3' + H_5'$). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.47 (isobutyl –CH–), 20.09 + 20.13 (isobutyl –CH_3), 29.50 (–CH–CH_3), 36.06 (isobutyl –CH_2–), 44.11 (–CH–CH_3), 46.32 (piperazine $C_2 + C_6$), 49.07 (piperazine $C_3 + C_5$), 69.28 (–N–CH_2–N–), 98.26 (phenyl C_4'–C≡N), 114.12 (phenyl $C_2' + C_6'$), 119.94 (phenyl C_4'), 126.93 (phenyl $C_3 + C_5$), 129.40 (phenyl $C_2 + C_6$), 133.29 (phenyl $C_3' + C_5'$), 136.73 (phenyl C_4), 140.51 (phenyl C_1), 152.90 (phenyl C_1'), 164.07 (C=N), and 177.71 (C=S). Anal. calcd. for $C_{26}H_{31}N_5OS$: C, 67.65, H, 6.77, N, 15.17, S, 6.95. Found: C, 67.70, H, 6.98, N, 15.20, S, 7.04.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2-cyanophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10o**)*

Compound **10o** was obtained as a white powder (52%). Mp: 80°C. IR ν_{max} (cm^{-1}): 2,945 (aromatic C–H), 2,837 (aliphatic C–H), 2,218 (C≡N), 1,617 (C=N), 1,594, 1,569, 1,510, 1,488 (aromatic C=C), 1,445 (C–N), 1,329 (C–O), 1,250 (C=S), 847 (1,4-dialkylphenyl C–H), and 765 (2-cyanophenyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.84 (6H, d, J = 6.8 Hz, isobutyl –CH_3), 1.57 (3H, d, J = 7.2 Hz, –CH–CH_3), 1.78–1.82 (1H, m, isobutyl –CH–), 2.41 (2H, d, J = 7.6 Hz, isobutyl –CH_2–), 2.90 (4H, t, J = 3.5 Hz, piperazine $H_2 + H_6$), 3.14 (4H, t, J = 3.5 Hz, piperazine $H_3 + H_5$), 4.37 (3H, q,

J = 7 Hz, –CH–CH_3), 5.03 (2H, s, –N–CH_2–N–), 7.10 (2H, t, J = 7.8 Hz phenyl H_5'), 7.15 (3H, d, J = 8 Hz phenyl $H_3 + H_5 + H_6'$), 7.22 (2H, d, J = 8 Hz, phenyl $H_2 + H_6$), 7.58–7.62 (1H, m, phenyl H_4'), and 7.70 (1H, dd, J = 7.6 Hz, J' = 1.6 Hz, phenyl H_3'). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.51 (isobutyl –CH–), 22.11 + 22.13 (isobutyl –CH_3), 29.49 (–CH–CH_3), 36.08 (isobutyl –CH_2–), 44.14 (–CH–CH_3), 49.66 (piperazine $C_2 + C_6$), 51.07 (piperazine $C_3 + C_5$), 69.38 (–N–CH_2–N–), 104.88 (phenyl C_2'–C≡N), 118.11 (phenyl C_6'), 119.19 (phenyl C_4'), 122.12 (phenyl C_2'), 126.96 (phenyl $C_3 + C_5$), 129.46 (phenyl $C_2 + C_6$), 134.18 (phenyl C_5'), 136.78 (phenyl C_4), 140.50 (phenyl C_1), 155.11 (phenyl C_1'), 164.15 (C═N), and 177.75 (C═S). Anal. calcd. for $C_{26}H_{31}N_5OS$: C, 67.65, H, 6.77, N, 15.17, S, 6.95. Found: C, 66.91, H, 6.79, N, 15.31, S, 7.09.

*5-[1-(4-Isobutylphenyl)ethyl]-3-{[4-(2-pyridyl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**10p**)*

Compound **10p** was obtained as a white powder (55%). Mp: 85°C. IR ν_{max} (cm^{-1}): 2,951 (aromatic C–H), 2,844 (aliphatic C–H), 1,618 (C═N), 1,591, 1,563, 1,512, 1,478 (aromatic C═C), 1,445 (C–N), 1,326 (C–O), 1,274 (C═S), 843 (1,4-dialkylphenyl C–H), and 774 (2-pyridyl C–H). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (6H, d, J = 6.4 Hz, isobutyl –CH_3), 1.54 (3H, d, J = 6.8 Hz, –CH–CH_3), 1.74–1.82 (1H, m, isobutyl –CH–), 2.42 (2H, d, J = 7.6 Hz, isobutyl –CH_2–), 2.78 (4H, t, J = 4.8 Hz, piperazine $H_2 + H_6$), 3.49 (4H, t, J = 4.8 Hz, piperazine $H_3 + H_5$), 4.32 (1H, q, J = 7.2 Hz, –CH–CH_3), 5.00 (2H, s, –N–CH_2–N–), 6.61–6.64 (1H, m, pyridyl H_5), 6.81 (1H, d, J = 8.4 Hz, pyridyl H_6), 7.11 (2H, d, J = 8 Hz, phenyl $H_3 + H_5$), 7.17 (2H, d, J = 8 Hz, phenyl $H_2 + H_6$), 7.49–7.53 (1H, m, pyridyl H_4), and 8.09 (1H, dd, J = 4.6 Hz, J' = 1.4 Hz, pyridyl H_3). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 18.45 (isobutyl –CH–), 22.11 + 22.13 (isobutyl –CH_3), 29.49 (–CH–CH_3), 36.06 (isobutyl –CH_2–), 44.12 (–CH–CH_3), 44.51 (piperazine $C_2 + C_6$), 49.34 (piperazine $C_3 + C_5$), 69.52 (–N–CH_2–N–), 107.17 (pyridyl C_6), 112.99 (pyridyl C_4), 126.95 (phenyl $C_3 + C_5$), 129.41 (phenyl $C_2 + C_6$), 136.74 (phenyl C_4), 137.45 (pyridyl C_5), 140.49 (phenyl C_1), 147.49 (pyridyl C_3), 159.32 (pyridyl C_1), 164.58 (C═N), and 178.22 (C═S). Anal. calcd. for $C_{24}H_{31}N_5OS$: C, 65.87, H, 7.14, N, 16.00, S, 7.33. Found: C, 65.32, H, 7.10, N, 16.12, S, 7.48.

4.2 | Biological evaluation

4.2.1 | Cell viability

The murine macrophage RAW264.7 cell line (American Type Culture Collection) was maintained in Dulbecco's modified Eagle's medium High Glucose supplemented with 10% fetal bovine serum and 1% penicillin (10,000 units/ml) and streptomycin (10,000 µg/ml) at 37°C under a 5% CO_2 atmosphere. Cell viability was measured by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, which depends on the mitochondria-based reduction of MTT formazan. Plated RAW 264.7 cells were treated with various concentrations of compounds (50–100 µM). After 24 hr, the cell medium was discarded and MTT solution (0.5 mg/ml) was added to wells for additional 2 hr at 37°C. After incubation, the cell culture medium was removed and 100 µl of isopropanol was used to dissolve formazan. The absorbance was determined at 570 nm wavelength by a microplate reader (Thermo Multiskan Spectrum, Finland). The absorbance of the control group was considered as 100%. The percentage of cell viability was calculated as follows:

$$\%\text{Viability} = \frac{(\text{Absorbance of treatment group} - \text{Absorbance of background})}{(\text{Absorbance of the control group} - \text{Absorbance of background})} \times 100\%.$$

4.2.2 | Anti-inflammatory activity

COX-1/COX-2 enzyme ınhibition assay

The inhibitory COX activity of the synthesized compounds was assayed using the Cayman COX fluorescent assay kit (Cayman No: 700100; Cayman Chemicals, Ann Arbor, MI) according to manufacturer's instructions. Each test compound was analyzed in duplicate applications. The enzyme-free and inhibitor-free assay systems were used as control experiments. The percentage of enzyme inhibition was calculated as follows:

$$\%\text{Inhibition} = \frac{(\text{Initial activity} - \text{Sample activity})}{(\text{Initial activity})} \times 100\%.$$

NO inhibition assay

The anti-inflammatory activity of compounds was evaluated by measuring the stable NO metabolite, nitrite, levels in cell culture media, with Griess reagent. RAW264.7 cells were plated, with a density of 1×10^6/ml, into a 48-well plate and incubated for 24 hr at 37°C in 5% CO_2. After cell culture medium was aspirated, cells were pretreated with various concentrations of compounds (50–100 µM) for 2 hr and then stimulated with 1 µg/ml of LPS (lipopolysaccharide from *Escherichia coli* 0111:B4; Sigma-Aldrich) for additional 22 hr. The collected culture supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 5% phosphoric acid) in a 96-well plate and incubated at room temperature for 10 min in the dark. The absorbance was determined using a microplate reader (Multiskan Ascent, Finland) at 540 nm wavelength. The concentration of nitrite in samples was calculated by using sodium nitrite standard curve. Indomethacin (100 µM) was used as a positive control.[25,26]

PGE_2 ınhibition assay

PGE_2 concentrations in cell culture supernatants of the compounds that have shown to reduce inflammation in NO assay were measured by using a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (Abcam PGE_2 ELISA Kit, UK) according to manufacturer's instructions.

4.2.3 | Statistical analysis

All repeated experiments were conducted in triplicate. Statistical analysis was performed by using GraphPad Prism 6 (Version 6.01; GraphPad

Software, Inc., San Diego, CA). Differences between groups were analyzed by using one-way analysis of variance, followed by Tukey's post-hoc tests. The statistical correlation was measured by Pearson's coefficient. Group differences were considered to be significant at $p < 0.05$.

4.3 | Docking studies

Docking studies were carried out by GLIDE,[26] as a standard docking program for the targets of COX-1 (PDB ID: 5U6X[22]) and COX-2 (PDB ID: 5IKT[23]) enzymes. To test whether the docking program can correctly reproduce the binding mode and to evaluate docking program, redocking experiments were performed using the cocrystallized inhibitors and the crystal structures. Glide was tested and Glidescore (SP) was chosen as the fitness function. The proper pose was evaluated according to RMSD of predicted conformations versus the cocrystallized ligand, based on the principle of docking poses with RMSD of <2.0 Å are in agreement with the X-ray structure. Therefore, this docking program and setup can be used in further studies. Furthermore, a data set of total 16 samples was generated. For this data set, 25 conformers for each molecule were produced using ConfGen.[27]

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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REFERENCES

[1] U. Weiss, *Nature Insight* **2008**, *454*, 427.
[2] R. Medzhitov, *Cell* **2010**, *140*, 771.
[3] O. Takeuchi, S. Akiri, *Cell* **2010**, *140*, 805.
[4] R. Medzhitov, *Nature* **2008**, *454*, 428.
[5] K. R. A. Abdellatif, M. A. Abdelgawad, H. A. H. Elshemy, S. S. R. Alsayed, *Bioorg. Chem.* **2016**, *64*, 1.
[6] B. J. Al-Hourani, S. K. Sharma, J. Y. Mane, J. Tuszynski, V. Baracos, T. Kniess, M. Suresh, J. Pietzsch, F. Wuest, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1823.
[7] M. C. Allison, A. G. Howatson, C. J. Torrance, F. D. Lee, R. I. Russell, *N. Engl. J. Med.* **1992**, *327*, 749.
[8] F. Halter, K. D. Rainsford, S. P. Sirko, A. Schmassmann, *Yale J. Biol. Med.* **1997**, *70*, 33.
[9] M. Colasanti, E. Cavalieri, T. Persichini, V. Mollace, S. Mariotto, H. Suzuki, G. M. Lauro, *J. Biol. Chem.* **1997**, *272*, 7582.
[10] K. Suzuki, H. Araki, H. Mizoguchi, O. Furukawa, K. Takeuchi K, *Digestion* **2001**, *63*, 92.
[11] K. Yokotani, Y. Okuma, Y. Osumi, *Br. J. Pharmacol.* **1996**, *117*, 653.
[12] S. Bala, S. Kamboj, V. Saini, D. N. Prasad, *J. Chem* **2013**, *2013*, 1. https://doi.org/10.1155/2013/412053
[13] T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang, P. C. Isakson, *J. Med. Chem.* **1997**, *40*, 1347.
[14] M. J. Langman, *JAMA* **1999**, *282*, 1929.
[15] A. Dallob, C. J. Hawkey, H. Greenberg, N. Wight, P. de Schepper, S. Waldman, P. Wong, L. deTora, B. Gertz, N. Agrawal, J. Wagner, K. Gottesdiener, *J. Clin. Pharmacol.* **2003**, *43*, 573.
[16] J. J. Talley, D. L. Brown, J. S. Carter, M. J. Graneto, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, R. S. Rogers, A. F. Shaffer, Y. Y. Zhang, B. S. Zweifel, K. Seibert, *J. Med. Chem.* **2000**, *43*, 775.
[17] L. Laine, *Semin. Arthritis Rheum.* **2002**, *32*, 25.
[18] K. Manjunatha, B. Poojary, P. L. Lobo, J. N. Fernandes, S. Kumari, *Eur. J. Med. Chem.* **2010**, *45*, 5225.
[19] A. S. Aboraia, H. M. Abdel-Rahman, N. M. Mahfouz, M. A. El-Gendy, *Bioorg. Med. Chem.* **2006**, *14*, 1236.
[20] M. J. Uddin, A. V. Elleman, K. Ghebreselasie, C. K. Daniel, B. C. Crews, K. D. Nance, T. Huda, L. J. Marnett, *ACS Med. Chem. Lett.* **2014**, *5*, 1254.
[21] V. Limongelli, M. Bonomi, L. Marinelli, F. L. Gervasio, A. Cavalli, E. Novellino, M. Parrinello, *PNAS* **2010**, *107*, 5411.
[22] G. Cingolani, A. Panella, M. G. Perrone, P. Vitale, G. Di Mauro, C. G. Fortuna, R. S. Armen, S. Ferorelli, W. L. Smith, A. Scilimati, *Eur. J. Med. Chem.* **2017**, *138*, 661.
[23] B. J. Orlando, M. G. Malkowski, *J. Biol. Chem.* **2016**, *291*, 15069.
[24] C. A. Harman, M. V. Turman, K. R. Kozak, L. J. Marnett, W. L. Smith, R. M. Garavito, *J. Biol. Chem.* **2007**, *282*, 28096.
[25] A. K. Kiemer, A. M. Vollmar, *Endocrinology* **1997**, *138*, 4282.
[26] R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin, D. T. Mainz, *J. Med. Chem.* **2006**, *49*, 6177.
[27] K. S. Watts, P. Dalal, R. B. Murphy, W. Sherman, R. A. Friesner, J. C. Shelley, *J. Chem. Inform. Modeling* **2010**, *50*, 534.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ozyazici T, Gurdal EE, Orak D, et al. Synthesis, anti-inflammatory activity, and molecular docking studies of some novel Mannich bases of the 1,3,4-oxadiazole-2(3*H*)-thione scaffold. *Arch Pharm*. 2020;e2000061. https://doi.org/10.1002/ardp.202000061