Received: 11 December 2020 | Revised: 13 April 2021 | Accepted: 16 April 2021

DOI: 10.1002/ardp.202000469

FULL PAPER

ARCH PHARM DPhG
Archiv der Pharmazie

Design, synthesis, and molecular docking of novel 3,5-disubstituted-1,3,4-oxadiazole derivatives as iNOS inhibitors

Meric Koksal[1] | Ayca Dedeoglu-Erdogan[1] | Marwa Bader[1,2] | Enise E. Gurdal[1,3] | Wolfgang Sippl[3] | Rengin Reis[4,5] | Melda Ozgurbuz[6] | Hande Sipahi[4] | Turgay Celik[6]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Omar Al-Mukhtar University, Al Bayda, Libya

[3]Department of Pharmaceutical Chemistry and Clinical Pharmacy, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

[4]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey

[5]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Acibadem University, Istanbul, Turkey

[6]Department of Pharmacology, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey

Correspondence
Meric Koksal, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University, Atasehir, 34755 Istanbul, Turkey.
Email: merickoksal@yeditepe.edu.tr

Abstract

To obtain new anti-inflammatory agents, recent studies have aimed to replace the carboxylate functionality of nonsteroidal anti-inflammatory drugs with less acidic heterocyclic bioisosteres like 1,3,4-oxadiazole to protect the gastric mucosa from free carboxylate moieties. In view of these observations, we designed and synthesized a series of 3,5-disubstituted-1,3,4-oxadiazole derivatives as inhibitors of prostaglandin E_2 (PGE_2) and NO production with an improved activity profile. As initial screening, and to examine the anti-inflammatory activities of the compounds, the inhibitions of the productions of lipopolysaccharide-induced NO and PGE_2 in RAW 264.7 macrophages were evaluated. The biological assays showed that, compared with indomethacin, compounds **5a**, **5g**, and **5h** significantly inhibited NO production with 12.61 ± 1.16, 12.61 ± 1.16, and 18.95 ± 3.57 μM, respectively. Consequently, the three compounds were evaluated for their in vivo anti-inflammatory activities. Compounds **5a**, **5g**, and **5h** showed a potent anti-inflammatory activity profile almost equivalent to indomethacin at the same dose in the carrageenan-induced paw edema test. Moreover, the treatment with 40 mg/kg of **5h** produced significant anti-inflammatory activity data. Furthermore, docking studies were performed to reveal possible interactions with the inducible nitric oxide synthase enzyme. Docking results were able to rationalize the biological activity data of the studied inhibitors. In summary, our data suggest that compound **5h** is identified as a promising candidate for further anti-inflammatory drug development with an extended safety profile.

KEYWORDS
1,3,4-oxadiazole, anti-inflammatory, docking study, nitric oxide, PGE_2

1 | INTRODUCTION

Inflammation is a protective physiological response of the immune system to infectious agents. Nevertheless, the inflammatory response may cause considerable damage to the cell, because microbial factors such as lipopolysaccharide (LPS) stimulate proinflammatory cytokines including inducible nitric oxide synthase (iNOS) and cyclooxygenases. Thus, as an inflammation response, a large amount of prostaglandin E_2 (PGE_2) and nitric oxide (NO) is produced in the immune cell.[1,2] Due to their potent vasodilator properties, NO and PGE_2 may cause classic inflammation symptoms like edema and redness. In addition, reacting with the superoxide anion, NO can be converted to peroxynitrite, which is an oxidizing molecule related to cellular damage.[3-5] By acting on neurons, PGE_2 also improves the systemic response toward inflammation including pain and hypersensitivity.[6-9] For this reason, as well as anti-inflammatory activity, PGE_2 is also an essential target for analgesic activity. However, as PGE_2 was found to be an immunosuppressant, it may contribute to the progression of inflammation and lead to severe diseases like cancer.[10] So, the importance or inhibition of PGE_2 synthesis has been increased in inflammatory and malign diseases such as cancer and rheumatoid arthritis.

In biological condition, NO is produced from L-arginine by nitric oxide synthase (NOS) in the presence of NADPH, molecular oxygen, and other cofactors. There are three isoforms of NOS in mammalian tissues named according to their activity or the tissue type in which they were first described.[11] Two of the isoforms, neuronal NOS and endothelial NOS, are constitutively expressed in mammalian cells and synthesize NO in response to an increase in intracellular Ca^{2+} levels for nerve function and blood pressure regulation, respectively. The third isoform, iNOS, activity is independent of the level of calcium in the cell and could be activated by the stimulation of cytokines or bacterial LPS. In addition, the production of NO by iNOS lasts much longer than from the other isoforms of NOS and tends to be in much higher and cytotoxic concentrations in the cell that can mediate inflammation and an innate immune response.[11-13]

Over the past several decades, many scientists have focused on the discovery of new nonsteroidal anti-inflammatory drugs (NSAIDs) with improved therapeutic profile and less side effects.[14,15] Molecular and conceptual models of inflammation have kept on evolving to bring new insight to generate novel therapeutic approaches for inflammatory diseases. Management of NO and PGE_2 production has become the current research strategy to develop new anti-inflammatory drugs. In a chemical manner, to obtain new anti-inflammatory agents, recent studies have aimed to replace the carboxylate functionality of NSAIDs with many types of less acidic heterocyclic bioisosteres like thiazole,[16] pyrazole,[17] and oxadiazole[18] to protect the gastric mucosa from free carboxylate moiety. During recent years, the compounds containing 1,3,4-oxadiazole core have been documented as remarkable anti-inflammatory agents.[19] Although salicylic acid and its properties are well known since decades and this compound is rather a weak anti-inflammatory agent, the results showed in this study indicate that introduction of 1,3,4-oxadiazole moiety and arylpiperazine/piperidine pharmacophore to its structure enhances its biological activity. In our previous study, the anti-inflammatory activity and gastric ulcerogenic potential of the 5-(3,4-dichlorophenyl)-3-[(4-substitutedpiperazin-1-yl)methyl]-1,3,4-oxadiaxole-2(3*H*)-thiones derivatives were investigated. These results showed that these 3,5-disubstituted-1,3,4-oxadiazole-2(3*H*)-thione derivatives showed the most prominent anti-inflammatory activity with low gastric ulceration incidence than the reference drug indomethacin.[20] In view of these observations and in continuation to our previous studies,[20-22] we have designed and synthesized a series of novel 3,5-disubstituted-1,3,4-oxadiazole derivatives as inhibitors of PGE_2 and NO production with an improved activity profile. All the synthesized and structurally confirmed compounds were screened in terms of their ability to inhibit the production of NO and PGE_2 with LPS-induced RAW 264.7 macrophages cells. In addition, in vivo anti-inflammatory assay was performed for three active compounds that have significant inhibitions in in vitro assays. Docking studies were carried out to rationalize the protein–ligand interactions between the target iNOS and the developed inhibitors.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Scheme 1 depicts the synthesis pathway of targeted 3,5-disubstituted-1,3,4-oxadiazole-2-thione derivatives (**5a–h**). The synthetic pathway was started with Fischer esterification of salicylic acid (**1**) and the resulting ester (**2**) was converted to acid hydrazide in the presence of hydrazine hydrate (**3**). The produced hydrazide group was cyclized with carbon disulfide and potassium hydroxide to obtain 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione ring (**4**).[22] For the last step, by using the Mannich reaction procedure, piperidine or piperazine derivatives and oxadiazole ring were linked with methylene chain (**5a–h**). The obtained compounds were purified by recrystallization with ethanol or salt formation.

Structural properties of purified compounds are given in Table 1. The newly synthesized compounds were characterized by infrared (IR), ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and elemental analysis. All of the compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structure. In general, the IR spectrum of all compounds exhibited characteristics peaks of free hydroxyl groups at 3320–3473 cm^{-1}, at 1610–1630 cm^{-1} for the C═N group, and 1435–1490 cm^{-1} for the C═S group. In the ^{1}H-NMR spectra, the hydroxyl groups of synthesized compounds appeared at δ 10.51–10.66. All the aromatic protons were obtained in the range of δ 7.00–7.65. The hydrogens of methylene bridge (N–$\underline{CH_2}$–N) of the compounds appeared at δ 5.00–5.07, and the piperazine or piperidine protons were observed in the range of δ 1.58–3.48. The ^{13}C-NMR spectra represent the signal of thione (C═S) group at δ 176.90–177.03 and the aromatic protons in the range of δ 108.80–157.98. The carbon of the

SCHEME 1 Synthetic pathway of the 3,5-disubstituted-1,3,4-oxadiazole derivatives (**5a–h**)

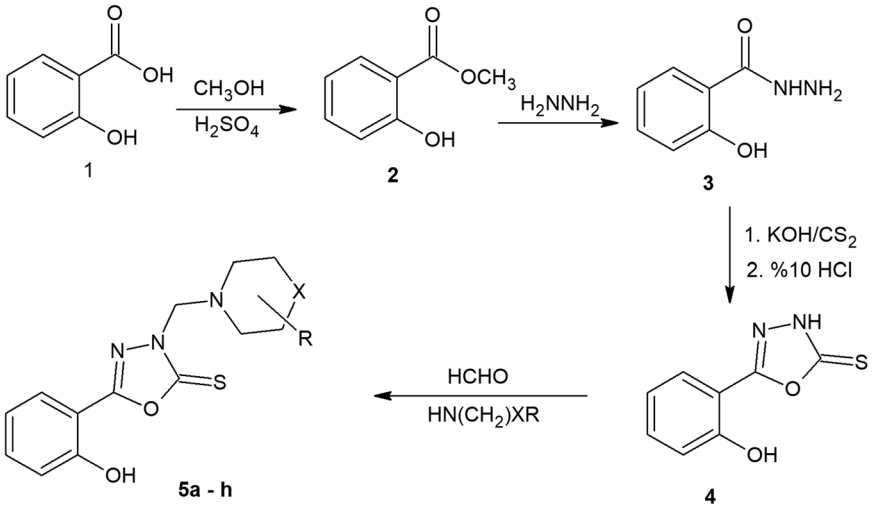
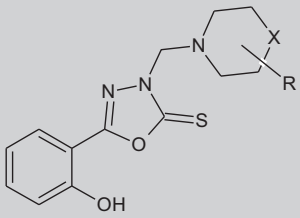


TABLE 1 Structural properties of the synthesized compounds



Compounds	–X	–R	Molecular formula	MW (g/mol)	mp (°C)	Yield (%)
5a	CH_2	3-Methyl	$C_{15}H_{19}N_3O_2S.H_2O$	323.41	>300	40
5b	CH	4-Hydroxymethyl	$C_{15}H_{19}N_3O_3S$	321.39	169.1	72
5c	CH	4-Phenyl	$C_{20}H_{21}N_3O_2S$	419.92	166.2	43
5d	C	4-Hydroxy-4-phenyl	$C_{20}H_{21}N_3O_3S.HCl$	445.96	251.1	42
5e	C	4-Acetyl-4-phenyl	$C_{22}H_{23}N_3O_3S.HCl$	428.93	154.7	53
5f	C	4-Cyano-4-phenyl	$C_{21}H_{20}N_4O_2S.HCl$	392.47	183.8	52
5g	N	4-Phenyl	$C_{19}H_{20}N_4O_2S$	368.45	172	42
5h	N	4-(2-Pyridyl)	$C_{18}H_{19}N_5O_2S$	369.44	147.4	59

Abbreviations: MW, molecular weight, mp, melting point.

methylene bridge (N–$\underline{CH_2}$–N) was at δ 69.62–70.56. The ^{1}H-NMR, ^{13}C-NMR, and elemental analysis results of the compounds are given in the Supporting Information.

2.2 | Pharmacology

2.2.1 | In vitro anti-inflammatory activity

Cell cytotoxicity

To examine the anti-inflammatory activities of the target compounds, the inhibitions of the productions of LPS-induced NO and PGE_2 in RAW 264.7 macrophages were evaluated. The cytotoxic effects of compounds on RAW 264.7 macrophages were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The assay results of synthesized compounds that show data recorded for these doses will not be due to cytotoxicity against RAW 264.7 cells are summarized in Table 2. The results indicated that compounds did not inhibit 50% of RAW 264.7 macrophages growth up to 100 μM except for **5d**. For compound **5d**, IC_{50} was found to be 37.92 ± 9.10 μM, and therefore nitrite assay was performed at 25 μM, which was determined to be a safe dose (Table 2). Indomethacin, known as a powerful anti-inflammatory agent, was used as the reference drug for comparisons in all biological studies.

NO and PGE_2 production inhibition assays

As an initial screening, we tested the inhibitory capacity of synthesized compounds against NO production at their nontoxic

TABLE 2 Cytotoxicity, nitrite, and prostaglandin E_2 (PGE_2) levels in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells treated with compounds

Entry	Dose (μM)	Cytotoxicity (IC_{50} values) (μM ± *SD*)	Nitrite level (μM ± *SD*)	PGE_2 production (pg/ml)
Control			0.17 ± 0.049	200.97 ± 79.16
Control + LPS			39.42 ± 2.03	4157.77 ± 402.71
5a	50	-	25.30 ± 3.66*	-
	100	>100	12.61 ± 1.16**	3754.05 ± 53.50
5b	100	>100	29.05 ± 2.32	3918.12 ± 163.06
5c	100	>100	34.23 ± 0.18	3677.78 ± 25.52
5d	25	37.92 ± 9.10	35.13 ± 0.38	
5e	100	>100	31.37 ± 1.79	3854.27 ± 46.72
5f	100	>100	24.38 ± 0.54*	3702.56 ± 52.50
5g	50	-	36.30 ± 0.51	-
	100	>100	11.06 ± 1.34**	3975.56 ± 220.50
5h	50	-	37.99 ± 2.90	-
	100	>100	18.95 ± 3.57*	3670.08 ± 22.55
Indomethacin	100	>100	19.08 ± 1.34*	266.67 ± 15.28**

Note: The significant differences between groups and control + LPS were defined with $^*p < .05$ and $^{**}p < .01$.

doses in LPS-induced RAW 264.7 cells, a murine macrophage cell line that is commonly used in screening for the identification of anti-inflammatory compounds.[23,24] As given in Table 2, compounds **5a–h** expressed varying inhibitory activities against LPS-induced NO productions at tested concentrations. Interestingly, the compounds possessing piperazine ring exhibited higher NO production inhibition than reference indomethacin, whereas the others with piperidine ring except compound **5a** showed weak activities. More specifically, compound **5g** showed the most potent inhibitory activity against NO production with 72.01 ± 3.97%, which is lower than the reference value, whereas the piperidine analog of this derivative almost did not cause any inhibition. Moreover, the compounds carrying disubstitution on the fourth position of piperidine did not exhibit significant activities at the tested concentration. On the basis of the in vitro anti-inflammatory model results illustrated in Table 2, the three compounds (**5a**, **5g**, and **5h**) exerted higher NO inhibition capacity as compared with the reference drug in 100 μM dose. In addition, when **5a** is tested at 50 μM, dose-dependent nitrite inhibition can be seen with this compound.

Inhibitor activities of all synthesized compounds were additionally tested with a PGE_2 assay kit technique. According to PGE_2 test results, the compounds lead to a slight decrease in PGE_2 levels, which was statistically insignificant (Table 2). It is noticeable that the compounds are more active as inhibitors of NO production rather than PGE_2 production. According to in vitro biological assays, compounds **5a**, **5g**, and **5h** that exhibited the most potent inhibitory activities at 100 μM were selected for in vivo studies to identify the lead compound of this group. Furthermore, the favorable in vitro anti-inflammatory activities of the compounds led us to molecular docking to evaluate the probable interactions of these active compounds with iNOS protein.

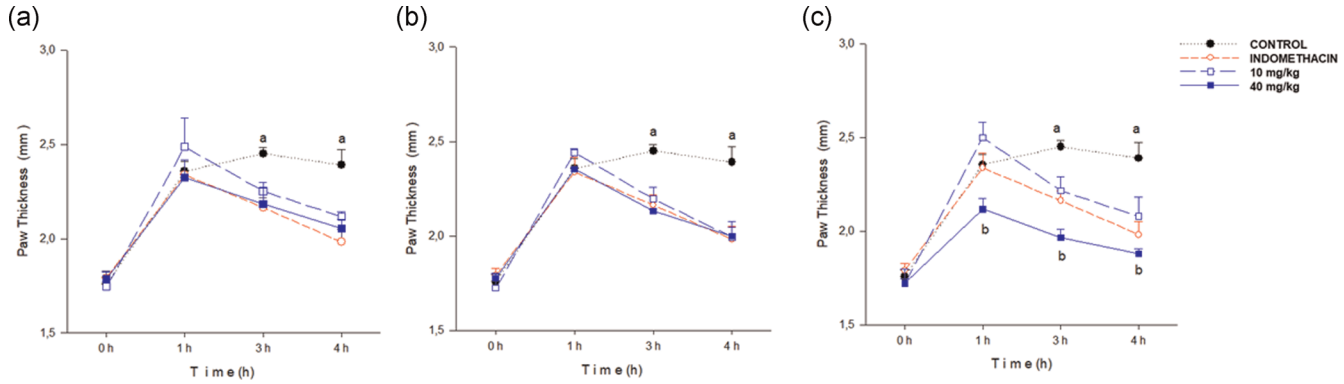


FIGURE 1 The effects of **5a** (a), **5g** (b), and **5h** (c) on carrageenan-induced paw edema in mice ($n = 6$). Data are the mean ± SEM. (a) $p \leq .05$, compared with vehicle (two-way analysis of variance [ANOVA] followed by Tukey's post-hoc test). (b) $p \leq .05$, compared with indomethacin (positive standard) (one-way ANOVA followed by Tukey's post-hoc test)

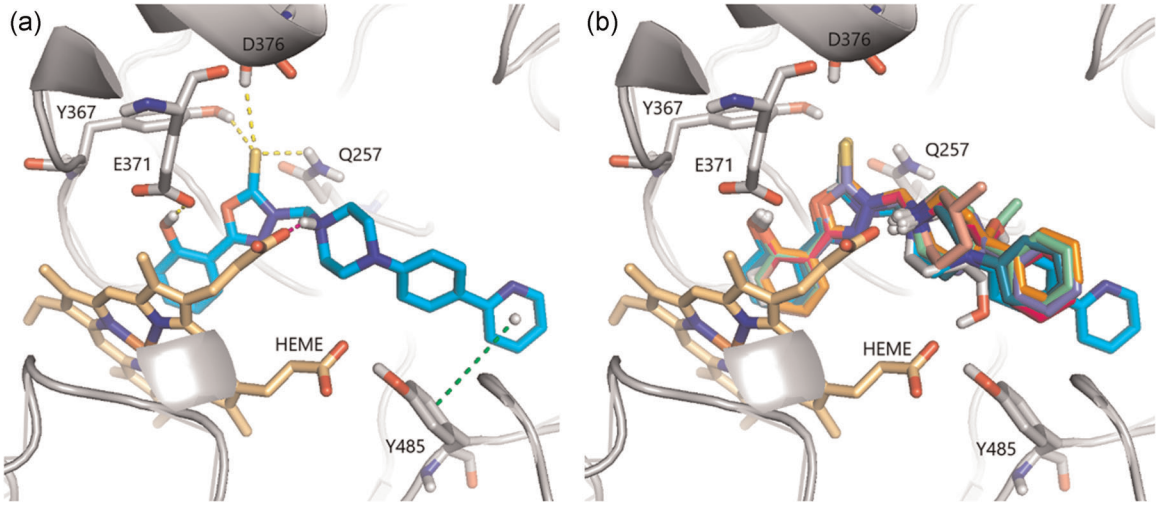


FIGURE 2 Docking poses of **5h** (a) and developed inhibitors (b) on inducible nitric oxide synthase enzyme (PDB ID: 1R35)

2.2.2 | In vivo anti-inflammatory activity

To evaluate the in vivo anti-inflammatory potency of selected derivatives, **5a**, **5g**, and **5h**, carrageenan-induced paw edema test as an acute inflammation animal model was performed.[25] The anti-inflammatory effects of compounds are demonstrated in Figure 1, separately. Oral administrations of compounds **5a**, **5g**, and **5h** both at 10- and 40-mg/kg doses exhibited a significant decrease in paw edema. As a positive control, indomethacin showed an inhibitory effect on edema development induction at the third and fourth hours. Treatment with compounds **5a** and **5g** in two applied doses yielded a similar inhibitory activity with a dose of 10 mg/kg indomethacin and also suppressed edema formation after edema induction at the third and fourth hours (Figure 1a,b). Although, the treatment with 10 mg/kg dose of 5h showed similiar activity profile such as 5a and 5g, 40 mg/kg dose oral administration of the compound exhibited significantly more inhibitory activity than reference indomethacin. Moreover, it suppressed significantly edema formation after edema induction at the first, third, and fourth hours (Figure 1c). As suggested above, the results of the acute inflammatory model indicated that compound **5h** has a potential as an anti-inflammatory agent against inflammatory edema.

TABLE 3 Docking scores of compounds (PDB ID: 1R35)

Compound	Docking score
5a_R	−6.426
5a_S	−6.718
5b	−6.758
5c	−6.615
5d	−6.675
5e	−6.948
5f	−6.957
5g	−6.938
5h	−6.724
Cocrystallized ligand	−5.304 (RMSD: 0.150)

Abbreviation: RMSD, root mean square deviation.

2.3 | Molecular docking studies

iNOS can be activated by cytokines or LPS, resulting in a great amount of NO secretion to cause inflammation and an innate immune response independent of Ca^{2+}.[26] To evaluate the possible interactions of the developed compounds, docking studies were performed with iNOS (PDB ID: 1R35[27]). Binding to iNOS requires the interaction with Glu377 that corresponds to Glu371 in murine iNOS that was used for the current biological study. Docking studies revealed a common binding mode of the developed compounds. In particular, the inhibitors form an H-bond between its phenol and a glutamate residue, a salt bridge between the protonated piperidine/piperazine, and a propionate group of protoporphyrin. In addition, an H-bond between the thioxo sulfur and Asp376 carboxylate was observed as a common interaction. The binding mode of the active inhibitor **5h** is depicted in Figure 2 as an example to represent the series. Equatorial conformation of bulky substituents at the piperidine was found to be more favorable in the docking studies. Accordingly, corresponding docking scores of all compounds are given in Table 3.

3 | CONCLUSION

In conclusion, a series of 3,5-disubstituted-1,3,4-oxadiazole-2-thione derivatives containing piperidine and piperazine fragment was synthesized. According to in vitro activity result, several compounds were displayed as potent inhibitors of NO production rather than inhibitors of PGE_2 release. Among them, compounds **5a**, **5g**, and **5h**

showed the highest activities as promising NO production inhibitors in LPS-induced RAW 264.7 macrophages with low cytotoxicity. These three compounds were selected for in vivo anti-inflammatory activity assay and molecular docking studies were performed on all compounds to evaluate their possible interactions with the iNOS active site. Docking studies demonstrated that the anti-inflammatory activities of the compounds are reasonable due to the fulfilling of necessary interactions with the iNOS enzyme, that is, hydrogen bonding to Glu371 and Asp376 and ionic interaction with protoporphyrin. According to in vivo acute inflammatory models, **5h** is a potential anti-inflammatory agent against inflammatory edema. Therefore, compound **5h** can be utilized as a promising lead compound for further development of new and safe anti-inflammatory agents.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All chemicals and reagents used in the present study were of analytical grade. The substituted piperazine and piperidine derivatives were ordered from chemical company Aldrich. The reactions were monitored by thin-layer chromatography on Merck precoated silica GF254 plates. Melting points were determined by using a Mettler Toledo FP62 capillary melting point apparatus (Mettler-Toledo) and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (version 5.0.1) (Perkin Elmer), using potassium bromide pellets, and the frequencies were expressed in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra (see the Supporting Information) were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian), using tetramethylsilane as the internal reference, with dimethylsulfoxide (DMSO) as a solvent, the chemical shifts were reported in parts per million (ppm), and coupling constants (*J*) were given in hertz (Hz). Elemental analyses were performed on LECO 932 CHNS instrument (Leco-932).

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 synthesis of 3,4-dichlorobenzohydrazide (**3**)

A solution of 2-hydroxybenzoic acid (salicylic acid) **1** (0.05 mol, 6.9 g), methanol (20 ml), and a catalytic amount of conc. H_2SO_4 was refluxed for 3 h. The reaction mixture was cooled and extracted with dichloromethane to obtain the pure methyl ester **2**.[28] The solution of methyl ester of salicylic acid (0.1 mol, 15.2 g) and 80% hydrazine hydrate (30 ml) in absolute ethanol (80 ml) was refluxed for 12–14 h. The excess solvent was distilled off and the concentrated solution was poured into ice water. The solid separated was filtered, washed, and dried; the crude product was purified by recrystallization from ethanol.[29]

4.1.3 | General procedure for the synthesis of 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (**4**)

A mixture of salicylic acid hydrazide **3** (0.05 mol, 7.5 g), potassium hydroxide (0.05 mol, 3 g), carbon disulfide (0.17 mol, 10 ml), and ethanol (70 ml) was stirred and refluxed for 12 h. The solvent was removed in vacuo and the residue was acidified with hydrochloric acid (2 mol/l). The resulting precipitate was filtered, washed with water, and recrystallized from ethanol.[21]

4.1.4 | General procedure for the synthesis of 5-(2-hydroxyphenyl)-3-substituted-1,3,4-oxadiazole-2(3*H*)-thiones (**5a–h**)

Formaldehyde solution 37% (0.02 mol, 1.5 ml) was added to a stirred solution of 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione **4** (4 g, 0.02 mol) in absolute ethanol (40 ml). An ethanolic solution (10 ml) of the appropriate amine (0.02 mol) was added portion-wise to the reaction mixture, stirred for 3 h at room temperature, and left overnight in a refrigerator. The crude products were filtered, washed with water, dried, and crystallized from appropriate solvents.[30]

*5-(2-Hydroxyphenyl)-3-[(3-methylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5a**)*[21]

FT-IR (KBr, cm^{-1}); 3473 (OH), 2950 (CH aromatic), 2811 (CH aliphatic), 1625 (C═N), 1572 (C═C), 1490 (C═S), and 1235 (C–N). ^{1}H-NMR (DMSO, ppm); 0.80 (d, 3H, $-CH_3$, J = 6.4 Hz), 1.59–1.40 (m, 4H, $H_4 + H_5$), 2.08 (t, 1H, piperidine H_3, J = 10.8 Hz), 2.39 (t, 2H, piperidine H_6, J = 12 Hz), 2.96 (d, 2H, piperidine H_3, J = 10.8 Hz), 5.04 (s, 2H, N–CH_2–N), 6.96 (t, 1H, 2-hydroxyphenyl H_5, J = 7.6 Hz), 7.02 (d, 1H, 2-hydroxyphenyl H_6, J = 8 Hz), 7.40–7.04 (m, 1H, 2-hydroxyphenyl H_4), 7.62 (dd, 1H, 2-hydroxyphenyl H_3, J = 8 Hz, J' = 2 Hz), and 10.54 (bs, 1H, OH). ^{13}C-NMR (DMSO, ppm); 40.05 (piperidine, $C_3 + C_5$), 49.96 (piperidine, $C_2 + C_6$), 70.49 (N–CH_2–N), 108.99 (2-hydroxyphenyl C_5), 116.94 (2-hydroxyphenyl C_4), 119.40 (2-hydroxyphenyl C_6), 128.79 (2-hydroxyphenyl C_3), 133.40 (2-hydroxyphenyl C_1), 156.28 (2-hydroxyphenyl C_2), 157.98 (oxadiazole C_5), and 177.01 (oxadiazole C═S). Analytical calculated for $C_{15}H_{19}N_3O_2S.H_2O$; Cal. C, 54.16; H, 4.31; N, 13.30; S, 7.61. Found: C, 54.10; H, 4.31; N, 13.43; S, 7.60.

*5-(2-Hydroxyphenyl)-3-[(4-hydroxymethylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5b**)*

FT-IR (KBr, cm^{-1}); 3469 (OH), 2938 (CH aromatic), 2810 (CH aliphatic), 1610 (C═N), 1573 (C═C), 1436 (C═S), and 1254 (C–N). ^{1}H-NMR (DMSO, ppm); 1.14–1.01 (m, 2H, H_5), 1.26–1.21 (m, 1H, H_4), 1.61 (d, 2H, piperidine H_3, J = 11.2 Hz), 2.42 (t, 2H, piperidine H_6, J = 11.6 Hz), 3.02 (d, 2H, $-CH_2OH$, J = 11.2 Hz), 3.21 (t, 2H, piperidine H_2, J = 5.6 Hz), 4.39 (t, 1H, OH), 5.00 (s, 2H, N–CH_2–N), 6.98–6.94 (m, 1H, 2-hydroxyphenyl H_5), 7.02 (d, 1H, 2-hydroxyphenyl H_6, J = 8.4 Hz), 7.43–7.39 (m, 1H, 2-hydroxyphenyl H_4), 7.62 (dd, 1H, 2-hydroxyphenyl H_3, J = 7.8 Hz, J' = 1.6 Hz), and 10.55 (bs, 1H, OH).

^{13}C-NMR (DMSO, ppm); 28.60 (piperidine, $C_3 + C_5$), 37.53 (piperidine, C_4), 50.03 (piperidine, $C_2 + C_6$), 70.56 (N–CH_2–N), 109.04 (2-hydroxyphenyl C_5), 117.02 (2-hydroxyphenyl C_4), 119.45 (2-hydroxyphenyl C_6), 128.39 (2-hydroxyphenyl C_3), 133.51 (2-hydroxyphenyl C_1), 156.35 (2-hydroxyphenyl C_2), 157.35 (oxadiazole C_5), and 177.03 (oxadiazole C=S). Analytical calculated for $C_{15}H_{19}N_3O_3S.H_2O$; Cal. C, 56.06; H, 5.96; N, 13.07; S, 9.98. Found: C, 55.98; H, 6.29; N, 13.02; S, 9.90.

*5-(2-Hydroxyphenyl)-3-[(4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5c**)*

FT-IR (KBr, cm^{-1}); 3469 (OH), 2959 (CH aromatic), 2817 (CH aliphatic), 1625 (C=N), 1592 (C=C), 1489 (C=S), and 1253 (C–N). ^{1}H-NMR (DMSO, ppm); 10.52 (bs, 1H, OH), 7.66 (dd, 1H, 2-hydroxyphenyl H_3, $J = 8$ Hz, $J' = 2$ Hz), 7.46–7.41 (m, 1H, 2-hydroxyphenyl H_4), 7.25–7.13 (m, 5H, phenyl), 7.04 (dd, 1H, 2-hydroxyphenyl H_6, $J = 8.4$ Hz, $J' = 0.8$ Hz), 6,99–6.95 (m, 1H, 2-hydroxyphenyl H_5), 5.07 (s, 2H, N–CH_2–N), 3.14 (d, 2H, piperidine H_2, $J = 12$ Hz), 2.60 (t, 2H, piperidine H_3, $J = 10.4$ Hz), 2.46–2.40 (m, 1H, piperidine H_4), 1.73 (d, 2H, piperidine H_6, $J = 10.8$ Hz), and 1.67–1.58 (m, 2H, piperidine H_5). ^{13}C-NMR (DMSO, ppm); 32.84 (piperidine, $C_3 + C_5$) 40.87 (piperidine, C_4), 50.57 (piperidine, $C_2 + C_6$), 70.33 (N–CH_2–N), 108.96 (2-hydroxyphenyl C_5), 117.00 (2-hydroxyphenyl C_4), 119.41 (2-hydroxyphenyl C_6), 125.92 (phenyl C_4), 126.57 (phenyl $C_2 + C_6$), 128.22 (phenyl $C_3 + C_5$), 129.02 (2-hydroxyphenyl C_3), 133.57 (2-hydroxyphenyl C_1), 145.98 (phenyl, C_1), 156.28 (2-hydroxyphenyl C_2), 157.85 (oxadiazole C_5), and 176.96 (oxadiazole C=S). Analytical calculated for $C_{20}H_{21}N_3O_2S$; Cal. C, 65.37; H, 5.76; N, 11.44; S, 8.73. Found: C, 64.64; H, 5.49; N, 11.44; S, 8.73.

*5-(2-Hydroxyphenyl)-3-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5d**)*

FT-IR (KBr, cm^{-1}); 3335 (OH), 2954 (CH aromatic), 2829 (CH aliphatic), 1625 (C=N), 1591 (C=C), 1489 (C=S), and 1252 (C–N). ^{1}H-NMR (DMSO, ppm); 1.74 (d, 2H, piperidine H_6, $J = 6.4$ Hz), 2.32–2.20 (m, 1H, piperidine H_4), 2.50 (t, 2H, piperidine H_3, $J = 3.8$ Hz), 3.22 (d, 2H, piperidine H_2, $J = 7.6$ Hz), 5.45 (s, 2H, N–CH_2–N), 7.48–7.24 (m, 5H, phenyl), 7.48–7.46 (m, 1H, 2-hydroxyphenyl H_4), 7.46 (d, 1H, 2-hydroxyphenyl H_3, $J = 2$ Hz), and 9.08 (bs, 2H, OH). Analytical calculated for $C_{20}H_{21}N_3O_3S$; Cal. C, 62.64; H, 5.52; N, 10.96; S, 8.36. Found: C, 62.71; H, 5.78; N, 10.45; S, 8.41.

*5-(2-Hydroxyphenyl)-3-[(4-acetyl-4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5e**)*

FT-IR (KBr, cm^{-1}); 3372 (OH), 2963 (CH aromatic), 2830 (CH aliphatic), 1799 (C=O), 1630 (C=N), 1596 (C=C), 1490 (C=S), and 1259 (C–N). ^{1}H-NMR (DMSO, ppm); 1.84 (s, 3H, CH_3), 1.99–1.93 (m, 2H, piperidine H_5), 2.43 (d, 2H, piperidine H_6, $J = 14$ Hz), 2.64 (t, 2H, piperidine H_3, $J = 9.6$ Hz), 2.94–2.91 (m, 2H, piperidine H_2), 5.00 (s, 2H, N–CH_2–N), 6,99–6.96 (m, 1H, 2-hydroxyphenyl H_5), 7.06 (d, 1H, 2-hydroxyphenyl H_6, $J = 8$ Hz), 7.56–7.43 (m, 1H, 2-hydroxyphenyl H_4), 7.47–7.24 (m, 5H, phenyl), 7.61 (dd, 1H, 2-hydroxyphenyl H_3, $J = 8$ Hz, $J' = 1$ Hz), and 10.56 (bs, 1H, OH). ^{13}C-NMR (DMSO, ppm); 25.47 (CH_3) 32.11 (piperidine, $C_3 + C_5$), 47.40 (piperidine, C_4), 53.50 (piperidine, $C_2 + C_6$), 69.98 (N–CH_2–N), 108.86 (2-hydroxyphenyl C_5), 117.03 (2-hydroxyphenyl C_4), 119.40 (2-hydroxyphenyl C_6), 126.22 (phenyl C_4), 126.97 (phenyl $C_2 + C_6$), 128.75 (phenyl $C_3 + C_5$), 129.02 (2-hydroxyphenyl C_3), 133.63 (2-hydroxyphenyl C_1), 141.26 (phenyl, C_1), 156.36 (2-hydroxyphenyl C_2), 157.83 (oxadiazole C_5), 176.92 (oxadiazole C=S), and 208.91 (C=O). Analytical calculated for $C_{22}H_23N_3O_3S.HCl$; Cal. C, 65.37; H, 5.76; N, 11.44; S, 8.73. Found: C, 64.64; H, 5.49; N, 11.44; S, 8.73.

*5-(2-Hydroxyphenyl)-3-[(4-cyano-4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5f**)*

FT-IR (KBr, cm^{-1}); 3363 (OH), 2952 (CH aromatic), 2832 (CH aliphatic), 2240 (C≡N), 1625 (C=N), 1597 (C=C), 1490 (C=S), and 1253 (C–N). ^{1}H-NMR (DMSO, ppm); 2.06–1.99 (m, 2H, piperidine H_5), 2.15 (d, 2H, piperidine H_6, $J = 12.8$ Hz), 2.85–2.83 (m, 2H, piperidine H_3), 3.25 (d, 2H, piperidine H_2, $J = 12.4$ Hz), 5.13 (s, 2H, N–CH_2–N), 7.00 (t, 1H, 2-hydroxyphenyl H_5, $J = 7.6$ Hz), 7.09 (d, 1H, 2-hydroxyphenyl H6, $J = 8.4$ Hz), 7.54–7.35 (m, 5H, phenyl), 7.65 (dd, 1H, 2-hydroxyphenyl H3, $J = 7.6$ Hz, $J' = 1.6$ Hz), and 10.61 (bs, 1H, OH). ^{13}C-NMR (DMSO, ppm); 35.12 (piperidine, $C_3 + C_5$), 41.29 (piperidine, C4), 47.52 (piperidine, $C_2 + C_6$), 69.62 (N–CH_2–N), 108.81 (2-hydroxyphenyl C_5), 117.06 (2-hydroxyphenyl C_4), 119.43 (2-hydroxyphenyl C_6), 121.60 (phenyl C_4), 125.52 (phenyl $C_2 + C_6$), 128.00 (2-hydroxyphenyl C_3), 128.93 (phenyl $C_3 + C_5$), 129.07 (2-hydroxyphenyl C_3), 133.74 (2-hydroxyphenyl C_1), 139.92 (phenyl, C_1), 156.46 (2-hydroxyphenyl C_2), 157.98 (oxadiazole C5), and 176.99 (oxadiazole C=S). Analytical calculated for $C_{20}H_{20}N_4O_2S.HCl$; Cal. C, 64.27; H, 5.14; N, 14.28; S, 8.17. Found: C, 63.86; H, 4.86; N, 14.37; S, 8.28.

*5-(2-Hydroxyphenyl)-3-[(4-phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5g**)*[21]

FT-IR (KBr, cm^{-1}); 3332 (OH), 2886 (CH aromatic), 2830 (CH aliphatic), 1626 (C=N), 1598 (C=C), 1489 (C=S), and 1223 (C–N). ^{1}H-NMR (DMSO, ppm); 2.90 (t, 4H, piperazine $H_2 + H_6$, $J = 4.8$ Hz), 3.13 (t, 4H, piperazine $H_3 + H_5$, $J = 4.8$ Hz), 5.1 (s, 2H, N–CH_2–N), 6.76 (t, 1H, phenyl H_4, $J = 7.2$ Hz), 6.90 (bd, 2H, phenyl $H_2 + H_6$, $J = 8.0$ Hz), 6.95–6.99 (m, 1H, 2-hydroxyphenyl H_5), 7.05 (bd, 1H, 2-hydroxyphenyl H_6, $J = 8.4$ Hz), 7.18 (t, 2H, phenyl $H_3 + H_5$, $J = 7.8$ Hz), 7.42–7.46 (m, 1H, 2-hydroxyphenyl H_4), 7.66 (dd, 1H, 2-hydroxyphenyl H_3, $J = 8$ Hz, $J' = 1.6$ Hz), and 10.51 (bs, 1H, OH).

*5-(2-Hydroxyphenyl)-3-[(4-(2-pyridyl)phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione (**5h**)*[21]

FT-IR (KBr, cm-1); 3435 (OH), 3012 (CH aromatic), 1612 (C=N), 1603, 1560 (C=C), 1484 (C=S), and 1264 (C–N). ^{1}H-NMR (DMSO, ppm); 2.48 (t, 4H, piperazine $H_2 + H_6$, $J = 5$ Hz), 3.48 (t, 4H, piperazine $H_3 + H_5$, $J = 5$ Hz), 5.08 (s, 2H, N–CH_2–N), 6.60–6.57 (m, 1H, pyridyl H_5), 6.79 (t, 1H, pyridyl H_6, $J = 8.8$ Hz), 6.93–6.79 (m, 1H, phenyl H_5), 7.01 (d, 1H, phenyl H6, $J = 8$ Hz), 7.44–7.40 (m, 1H, phenyl H_4), 7.50–7.46 (m, 1H, 2-hydroxyphenyl H_4), 7.63 (dd, 1H, 2-hydroxyphenyl H_6, $J = 8$ Hz, $J' = 1.6$ Hz), 8.05 (dd, 1H, 2-hydroxyphenyl H_3, $J = 4$Hz, $J' = 2$ Hz), and 10.51 (bs, 1H, OH).

^{13}C-NMR (DMSO, 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 (2-hydroxyphenyl C5), 117.00 (2-hydroxyphenyl C_4), 119.46 (2-hydroxyphenyl C_6), 129.11 (pyridyl C_6), 133.61 (2-hydroxyphenyl C_3), 137.38 (2-hydroxyphenyl C_1), 147.41 (pyridyl C_3), 156.36 (2-hydroxyphenyl C_2), 157.84 (pyridyl C_1), 158.77 (C=N), and 176.98 (oxadiazole C=S). Analytical calculated for $C_{18}H_{19}N_4O_3S$; Cal. 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.

4.2 | Pharmacological assays

4.2.1 | MTT assay

Cell viability was examined using the MTT assay. Plated RAW 264.7 cells were exposed to 25, 50, and 100 µM of compounds dissolved in DMSO. Only compound **5d** was applied as 25 and 50 µM due to lack of solubility in DMSO. After 24 h of incubation, MTT was added to all wells at 0.5 mg/ml of concentration and incubated for an additional 2 h at 37°C. After discarding the medium from plates, 100 µl of isopropanol was added to the wells. Absorbance of the MTT formazan was determined at 570 nm by a UV-spectrophotometric plate reader (Thermo Multiscan Spectrum). Viability was defined as the ratio (expressed as a percentage) of absorbance of the cells exposed to compounds to the cells treated with 0.5% DMSO (as control). All measurements were done in triplicates.

4.2.2 | Nitrite and PGE_2 assays

RAW 264.7 macrophages cells (ATCC) were maintained in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum and 1% streptomycin and penicillin at 37°C in 5% CO_2. Anti-inflammatory activity of the compounds was evaluated by measuring the stable NO metabolite, nitrite, with Griess assay.[31] Briefly, RAW-264.7 cells were plated in a 48-well plate and incubated for 24 h at 37°C in 5% CO_2. Plated cells were pretreated with the same concentrations in cell viability assay for 2 h and then stimulated with 1 µg/ml of LPS for additional 22 h. The culture supernatant (50 µl) was mixed with Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 5% phosphoric acid Mettler]) and incubated at room temperature for 10 min. The absorbance of the mixture was determined at 540 nm using a microplate reader (BioTek). The amount of nitrite in the test samples was calculated using sodium nitrite (Fluka Chemika) standard curve. As a positive control, 100 µM of indomethacin (Fluka Chemika) was used. Analgesic and anti-inflammatory activities were determined with Prostaglandin E_2 ELISA Kit (Abcam) according to the manufacturer's instruction by using cell supernatants of anti-inflammatory activity assay. Only doses that showed the highest nitrite inhibition activity (100 µM) for each molecule were used. SPSS was used for all statistical analyses. Data related to cell viability, anti-inflammatory activity, PGE_2 levels were analyzed by using *t* test. Differences were considered as significant at $p < .05$.

4.2.3 | In vivo anti-inflammatory activity assay

Animals

Adult male BALBc mice (25–35 g) were kept in standard animal housing conditions at the temperature of 23 ± 1°C and suitable humidity (55 ± 5%) with dark–light cycles (12/12 h). The animals were kept in home cages prepared with plexiglass (65 × 25 × 15 cm), receiving food and water ad libitum. The mice were acclimatized to laboratory conditions for 1 day before the experiments were carried out. The experimental protocol was approved by the Ethical Committee of Yeditepe University Experimental Medicine Research Institute (Decision number: 2019/10-12). The animals were fasted (food was withdrawn, not water) for 3 h before use and during the experiment. Six mice in one group were used in all sets of the study. The experiments were performed between 8:00 a.m. and 10:00 a.m. in a soundproof laboratory. As doses, 10 and 40 mg/kg of compounds **5a**, **5g**, **5h**, and 10 mg/kg of indomethacin were selected and the suspension was made in normal saline by 0.25% CMC. Control group was used as a standard drug and normal saline (0.5 ml) was used as a vehicle.

Carrageenan-induced mice paw thickness assay

Indomethacin and other compounds were orally given to the animals 30 min before carrageenan injection. Acute inflammation was produced via injection of 20 µl of 2% carrageenan into the subplantar region of the right hind paw of the mice.[32] The measurement of thickness was immediately done before subplantar injection, and 1, 3, 4 h thereafter, using a micrometer that was particularly modified for the measurement of thickness. The increase in paw thickness was calculated according to the formula[33]:

$$\text{Percentage of inhibition} = [(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}] / (V_t - V_0)_{\text{control}}] \times 100,$$

where $(V_t - V_0)_{\text{control}}$ is the difference in the size of paw in control mice and $(V_t - V_0)_{\text{treated}}$ is the difference in the size of paw in mice treated with the treatments.

4.3 | Molecular docking

Docking studies were performed on iNOS (PDB ID: 1R35[27]) enzyme by GLIDE,[34] as a standard docking program. At least one H-bond with Glu371 residue was defined as a constraint during the preparation of the grid file. To test if the docking program correctly reproduces the binding mode and to evaluate the docking program, redocking experiments were carried out using the cocrystallized inhibitors and the crystal structures. Glidescore (SP) was selected as fitness function after the analysis of the proper pose investigated according to the root mean square deviation (RMSD) of predicted conformations versus the related native one, considering the principle that docking poses with RMSD of less than 2.0 Å are in agreement with the X-ray structure. Therefore, this docking program and setup were used in further studies. Then, a dataset of eight samples was generated. For this dataset, 64 conformers for each

molecule were produced by ConfGen.[35] In the case of piperidine derivatives, both equatorial and axial conformations and all stereoisomers of **5a** were included in the conformer generation. The figures were rendered by using PyMOL (The PyMOL Molecular Graphics System, Version 1.8.4, Schrödinger, LLC).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interests.

ORCID

Meric Koksal http://orcid.org/0000-0001-7662-9364
Ayca Dedeoglu-Erdogan http://orcid.org/0000-0001-5936-5328
Enise E. Gurdal http://orcid.org/0000-0003-1064-8639
Wolfgang Sippl http://orcid.org/0000-0002-5985-9261
Rengin Reis http://orcid.org/0000-0002-3484-2201
Melda Ozgurbuz http://orcid.org/0000-0002-8899-0816
Hande Sipahi http://orcid.org/0000-0001-6482-3143
Turgay Celik http://orcid.org/0000-0003-2787-2485

REFERENCES

[1] L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, L. Zhao, *Oncotarget* **2018**, *9*, 7204. https://doi.org/10.18632/oncotarget.23208
[2] J. R. Vane, R. M. Botting, *Inflam. Res.* **1995**, *44*, 1.
[3] D. Salvemini, Z. Q. Wang, P. S. Wyatt, D. M. Bourdon, M. H. Marino, P. T. Manning, M. G. Currie, *Br. J. Pharmacol.* **1996**, *118*, 829. https://doi.org/10.1111/j.1476-5381.1996.tb15475.x
[4] J. Marks-Konczalik, S. C. Chu, *J. Biol. Chem.* **1998**, *273*, 22201. https://doi.org/10.1074/jbc.273.35.22201
[5] D. Salvemini, P. T. Manning, B. S. Zweifel, K. Seibert, J. Connor, M. G. Currie, P. Needleman, J. L. Masferrer, *J. Clin. Invest.* **1995**, *96*, 301. https://doi.org/10.1172/JCI118035
[6] E. Ricciotti, G. A. FitzGerald, *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 986. https://doi.org/10.1161/ATVBAHA.110.207449
[7] A. Zarghi, S. Arfaei, *Iran. J. Pharm. Res.* **2011**, *10*, 655.
[8] G. Dannhardt, W. Kiefer, *Eur. J. Med. Chem.* **2001**, *36*, 109. https://doi.org/10.1016/S0223-5234(01)01197-7
[9] T. A. Samad, A. Sapirstein, C. J. Woolf, *Trends Mol. Med.* **2002**, *8*, 390. https://doi.org/10.1016/S1471-4914(02)02383-3
[10] M. Nakanishi, D. W. Rosenberg, *Semin. Immunopathol.* **2013**, *35*, 123. https://doi.org/10.1007/s00281-012-0342-8
[11] W. K. Alderton, C. E. Cooper, R. G. Knowles, *Biochem. J.* **2001**, *357*, 593. https://doi.org/10.1042/0264-6021:3570593
[12] M. Colasanti, H. Suziki, *Trends Pharmacol. Sci.* **2000**, *21*, 249. https://doi.org/10.1016/s0165-6147(00)01499-1
[13] D. J. Struchr, J. Santolini, Z. Q. Wang, C. C. Wei, S. Adak, *J. Biol. Chem.* **2004**, *279*, 36167. https://doi.org/10.1074/jbc.R400017200
[14] A. H. Abdelazeem, S. A. Abdelatef, M. T. El-Saadi, H. A. Omar, S. I. Khan, C. R. McCurdy, S. M. El-Moghazy, *Eur. J. Pharm. Sci.* **2014**, *62*, 197. https://doi.org/10.1016/j.ejps.2014.05.025
[15] T. B. Tumer, F. C. Onder, H. Ipek, T. Gungor, S. Savranoglu, T. T. Tok, A. Celik, M. Ay, *Int. Immunopharmacol.* **2017**, *43*, 129. https://doi.org/10.1016/j.intimp.2016.12.009
[16] M. H. M. Helal, M. A. Salem, M. S. A. El-Gaby, *Eur. J. Med. Chem.* **2013**, *65*, 517. https://doi.org/10.1016/j.ejmech.2013.04.005
[17] A. A. Bekhit, T. Abdel-Aziem, *Bioorg. Med. Chem.* **2004**, *12*, 1935. https://doi.org/10.1016/j.bmc.2004.01.037
[18] E. Durgashivaprasad, G. Mathew, S. Sebastian, S. M. Reddy, J. Mudgal, G. K. Nampurath, *Indian J. Pharmacol.* **2014**, *46*, 521. https://doi.org/10.4103/0253-7613.140584
[19] S. V. Bhandari, K. G. Bothara, M. K. Raut, A. A. Patil, A. P. Sarkate, V. J. Mokale, *Bioorg. Med. Chem.* **2008**, *16*, 1822. https://doi.org/10.1016/j.bmc.2007.11.014
[20] M. Koksal, M. Yarim, A. Erdal, A. Bozkurt, *Drug. Res. (Stuttg)* **2014**, *64*, 66. https://doi.org/10.1055/s-0033-1353184
[21] T. Ozyazici, E. E. Gurdal, D. Orak, H. Sipahi, T. Ercetin, H. O. Gulcan, M. Koksal, *Arch. Pharm.* **2020**, *353*, e2000061. https://doi.org/10.1002/ardp.202000061
[22] M. Koksal, I. Ozkan-Dagliyan, T. Ozyazici, B. Kadioglu, H. Sipahi, A. Bozkurt, S. S. Bilge, *Arch. Pharm.* **2017**, *350*, 1700153. https://doi.org/10.1002/ardp.201700153
[23] J. An, H. H. Lee, J. S. Shin, H. S. Yoo, J. S. Park, S. H. Son, S. W. Kim, J. Yu, J. Lee, K. T. Lee, N. J. Kim, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2613. https://doi.org/10.1016/j.bmcl.2017.03.057
[24] M. M. G. El-Din, M. I. El-Gamal, M. S. Abdel-Maksoud, H. Lee, J. Choi, T. W. Kim, J. S. Shin, H. H. Lee, H. K. Kim, K. T. Lee, D. Baek, *Bioorg. Med. Chem. Lett.* **2020**, *30*, 126884. https://doi.org/10.1016/j.bmcl.2019.126884
[25] L. Ma, C. Xie, Y. Ma, J. Liu, M. Xiang, X. Ye, H. Zheng, Z. Chen, Q. Xu, T. Chen, J. Chen, J. Yang, N. Qiu, G. Wang, X. Liang, A. Peng, S. Yang, Y. Wei, L. Chen, *J. Med. Chem.* **2011**, *54*, 2060. https://doi.org/10.1021/jm1011534
[26] M. F. A. Mohamed, A. A. Marzouk, A. Nafady, D. A. El-Gamal, R. M. Allam, G. E. A. Abuo-Rahma, H. I. El Subbagh, A. H. Moustafa, *Bioorg. Chem.* **2020**, *105*, 104439. https://doi.org/10.1016/j.bioorg.2020.104439
[27] E. A. Hallinan, S. W. Kramer, S. C. Houdek, W. M. Moore, G. M. Jerome, D. P. Spangler, A. M. Stevens, H. S. Shieh, P. T. Manning, B. S. Pitzele, *Org. Biomol. Chem.* **2003**, *1*, 3527. https://doi.org/10.1039/b307563j
[28] D. Chavez-Flores, J. M. Salvador, *Tetrahedron: Asymmetry* **2012**, *23*, 237. https://doi.org/10.1016/j.tetasy.2012.02.014
[29] S. L. Gaonkar, K. L. Rai, B. Prabhuswamy, *Eur. J. Med. Chem.* **2006**, *41*, 841. https://doi.org/10.1016/j.ejmech.2006.03.002
[30] A. S. Aboraia, H. M. Abdel-Rahman, N. M. Mahfouz, M. A. El-Gendy, *Bioorg. Med. Chem.* **2006**, *14*, 1236. https://doi.org/10.1016/j.bmc.2005.09.053
[31] K. Buran, R. Reis, H. Sipahi H, F. E. Önen Bayram, *Arch. Pharm.* **2021**, e2000354. https://doi.org/10.1002/ardp.202000354
[32] P. M. Zadeh-Ardabili, S. K. Rad, *Biotechnol. Rep.* **2019**, *22*, e00341. https://doi.org/10.1016/j.btre.2019.e00341
[33] M. J. Sammons, P. Raval, P. T. Davey, D. Rogers, A. A. Parsons, S. Bingham, *Brain Res.* **2000**, *876*, 48. https://doi.org/10.1016/S0006-8993(00)02596-8
[34] 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. https://doi.org/10.1021/jm051256o
[35] K. S. Watts, P. Dalal, R. B. Murphy, W. Sherman, R. A. Friesner, J. C. Shelley, *J. Chem. Inf. Model.* **2010**, *50*, 534. https://doi.org/10.1021/ci1000

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: M. Koksal, A. Dedeoglu-Erdogan, M. Bader, E. E. Gurdal, W. Sippl, R. Reis, M. Ozgurbuz, H. Sipahi, T. Celik. Design, synthesis, and molecular docking of novel 3,5-disubstituted-1,3,4-oxadiazole derivatives as iNOS inhibitors. *Arch. Pharm.* **2021**, e2000469. https://doi.org/10.1002/ardp.202000469