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Short communication

Synthesis and analgesic and anti-inflammatory activities 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal) hydrazone derivatives

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ABSTRACT

In this study new 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted benzal)hydrazone **V** derivatives were synthesized as analgesic and anti-inflammatory agents. The structures of compounds were elucidated by spectral and elemental analysis. Compounds **Va**, **Vb** and **Vc** were exhibited more potent analgesic activity than ASA. Also these derivatives demonstrated anti-inflammatory activity as well as standard compound indomethacin. Side effects of the compounds were examined on gastric mucosa. None of the compounds showed gastric ulcerogenic effect compared with reference nonsteroidal anti-inflammatory drugs (NSAIDs). On the basis of available data, the structure–activity relationship of **V** derivatives was also discussed.

1. Introduction

Pain is a problem not solved by medicine and pain is the most common reason that patients seek advice from pharmacist and other professionals and represent important medical and economic cost for the community. According to the World Health Organization, 90% of diseases are associated with pain. Despite growing knowledge of endogenous nociceptive and antinociceptive systems, many pain syndromes like rheumatoid arthritis and certain advanced cancers are still no adequate treatment [1].

Currently available nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, indomethacin and naproxen exhibit gastric toxicity. Long-term use of these drugs has been associated with gastrointestinal (GI) ulceration, bleeding and nephrotoxicity [2]. The GI damage from NSAIDs is generally attributed to two factors, i.e. local irritation by the carboxylic acid moiety common to most NSAIDs (topical effect); and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis [3]. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting cyclooxygenases (COXs) [4]. The chronic use of NSAIDs may elicit appreciable GI toxicity. Therefore synthetic approaches based upon chemical modification of NSAIDs have been taken with the aim of improving their safety profile.

It is known that some pyrazolone derivatives like dipyrone and phenylbutazone possess analgesic and anti-inflammatory activities, but several side effects have limited the clinical use of these drugs. Pyridazinone derivatives which are related structurally to pyrazolone derivatives in the point of ring enlargement of pyrazolone to pyridazinone. A lot of 3(2*H*)-pyridazinone derivatives have been reported as analgesic and anti-inflammatory agents without gastrointestinal side effect. This is agreement with in our experience in the pyridazinone field [5–9]. Among the pyridazinone derivatives endowed with antinociceptive effects, Emorfazone has emerged the latter, which was launched in Japan, displays an interesting profile because its activity is not mediated by interaction with the prostaglandins system or by affinity for opioids receptors [10,11]. Many authors reported that pyridazinone derivatives bearing an arylpiperazine moiety at side chain on the lactam nitrogen of the ring had significant analgesic activity [12–20]. Moreover, they claimed that these derivatives exhibited better analgesic activity if they bear a carbon chain between the nitrogen atom of the lactam and amine component of the side chain [21]. These results led us to design new structurally related derivatives, keeping the 6-(substitute arylpiperazinyl)-3(2*H*)-piridazinone

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framework and modifying the substitutent on the 2 position of the pyridazinone ring. As part of a program aimed at developing simple and efficient syntheses of pharmacologically useful pyridazinones, we synthesized new 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted benzal)hydrazone **V** derivatives. The analgesic and anti-inflammatory activities were investigated for the title compounds utilizing the phenylbenzoquinone-induced writhing test (PBQ test) and the carrageenan-induced foot paw edema test (CPE test) respectively. All the compounds were also tested for the irritative and ulcerogenic action on gastric mucosa.

2. Chemistry

New 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal)hydrazone **V** derivatives were synthesized according to Scheme 1. Initially, nucleophilic displacement reaction of commercial 3,6-dichloropyridazine with arylpiperazines in ethanol afforded 3-chloro-6-substitutedpyridazines **I**. The physical and spectral properties of 3-chloro-6-substitutedpyridazine **I** were in accordance with the literature [22,23]. Therefore we carried out the next steps of the reaction without any further analysis. Synthesis procedure of all **II**, **III**, **IV** derivatives reported by us in our previous study [5,9,24]. Hydrolysis of 3-chloro-6-substituted pyridazines **I** was carried out upon heating in glacial acetic acid to afford 6-substituted-3(2*H*)-pyridazinone **II** derivatives. The formation of these compounds was confirmed by IR spectra of a C=O signal at about 1660 cm^{-1}. Esterification of pyridazinones **II** was performed using ethyl bromoacetate in the presence of potassium carbonate in acetone at reflux temperature [9]. 6-Substituted-3(2*H*)-pyridazinone-2-ylacetohydrazide **IV** derivatives were prepared by the reaction of ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate **III** derivatives with hydrazine hydrate in ethanol at room temperature [24]. Finally, the target 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal)hydrazone **V** derivatives were synthesized via condensation of acetohydrazides **IV** with *p*-substituted benzaldehydes. (Scheme 1). The physical data, yield, and molecular formula of all compounds are reported Table 1.

3. Pharmacology

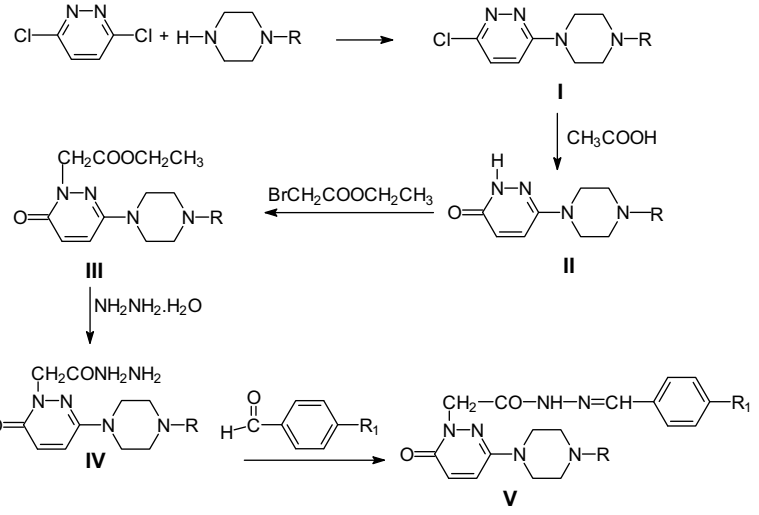
The analgesic activity of the compounds was studied by using phenylbenzoquinone-induced writhing test in mice [25]. In order to screen the anti-inflammatory activity of the synthesized compounds, carragenin-induced hind paw edema model in mice was used [26]. ASA and indomethacin were used as reference drugs in the tests respectively. All the pyridazinone derivatives have also been evaluated for acute toxicity and gastric ulcerogenic effect tests. The observed data on analgesic and anti-inflammatory activity of the compounds and reference drugs are given in Tables 2 and 3.

4. Results, discussions and conclusions

In this study, novel derivatives of 3(2*H*)-pyridazinone have been synthesized in attempt to find new derivatives having analgesic and anti-inflammatory activities. One of the most interesting characteristic of these novel compounds is their basic nature, which differentiates them from the classical acidic nonsteroidal anti-inflammatory agents (NSAIDs). It is interest, therefore to study analgesic-anti-inflammatory properties of these novel compounds.

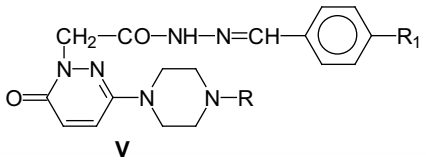
The synthesis pathway leading to the title compounds is given in Scheme 1. 6-Substituted-3-chloropyridazines **I** were synthesized in our laboratory according to reports in the literature [22,23]. As a starting material, 6-substituted-3(2*H*)-pyridazinone-2-yl acetohydrazide **IV** derivatives were used to produce new 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted benzal)hydrazone **V** derivatives. The structure of the compounds was elucidated by IR, ^{1}H NMR spectral data and elemental analysis. In the IR spectra of all new compounds, hydrazone C=O bands were observed at about 1682–1685 cm^{-1} region and lactam C=O bands were observed at about 1652–1660 cm^{-1} region. Furthermore, all of the **V** derivatives have N–H band at about 3172–3196 cm^{-1} region in their IR spectrum. The absorption bands associated with other functional groups appeared in the expected regions.

According to the literature [27,28], the hydrazones may exist as *Z*/*E* geometrical isomers about C=N double bonds and *cis*/*trans* amide conformers. It has been reported that when hydrazones are dissolved in dimethyl-d_6 sulfoxide solution, the *E* geometrical of these compounds undergo a rapid *cis*/*trans* amide equilibrium, in which the *cis* conformer predominates. ^{1}H NMR spectra of the 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted benzal)hydrazone **V** derivatives that are taken in DMSO d_6. In the ^{1}H NMR of the compounds **Va–i**, the signals belonging to benzylidene group were observed at aromatic region while signals belonging to the –$NHNH_2$ disappeared. Two sets of signals each belonging to the CH_2 and =CH group of *cis* and *trans* conformers were observed at 4.60–4.70/8.10–8.18 and 4.99–5.03/7.92–7.99 ppm. The upfield lines of =CH protons were assigned to *cis* conformer of the amide structure and downfield lines of the protons of the same group



Scheme 1. Synthesis of new 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal) hydrazone **V** derivatives.

Table 1
Physical constant of 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal)hydrazone **V** derivatives.



V

Com.	R	R_1	Yield (%)	Mp (°C)	Molecular formula (M. W)
Va	3-Chlorophenyl	H	67	194	$C_{23}H_{23}ClN_6O_2$
Vb	4-Chlorophenyl	H	70	191	$C_{23}H_{23}ClN_6O_2$
Vc	2-pyridyl	H	73	196	$C_{22}H_{23}N_7O_2$
Vd	3-Chlorophenyl	CH_3	62	168	$C_{24}H_{25}ClN_6O_2$
Ve	4-Chlorophenyl	CH_3	69	159	$C_{24}H_{25}ClN_6O_2$
Vf	2-pyridyl	CH_3	72	241	$C_{23}H_{25}N_7O_2$
Vg	3-Chlorophenyl	OCH_3	61	211	$C_{24}H_{25}ClN_6O_3$
Vh	4-Chlorophenyl	OCH_3	56	171	$C_{24}H_{25}ClN_6O_3$
Vi	2-pyridyl	OCH_3	64	165	$C_{23}H_{25}N_7O_3$

Melting points were determined on electrothermal melting point apparatus and uncorrected. The C, H, N were performed at Scientific and Technical Council of Turkey, Instrumental Analysis Center and within ±0.4% of the theoretical values.

were assigned to *trans* conformer of the amide structure [27]. NH proton of the hydrazone moiety was seen as singlet at about 11.28–11.34 ppm. All the other aromatic and aliphatic protons were observed at the expected region. Further spectroscopic details of these compounds are presented in the Experimental part.

All the newly synthesized compounds were tested for anti-inflammatory and analgesic activities. These results of anti-inflammatory and analgesic activities of all compounds are statistically significant. Analgesic activity evaluation was carried out using phenylquinone-induced writhing assay. 6-[4-(3-Chlorophenyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-benzal hydrazone **Va**, 6-[4-(4-chlorophenyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-benzal hydrazone **Vb** and 6-[4-(pyridyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-benzal hydrazone **Vc** derivatives have been shown better analgesic activity than reference compound ASA. These compounds have no substituent on benzal hydrazone aromatic ring. While the compounds **Va**, **Vb**, **Vc** exhibited remarkable analgesic activity, 6-[4-(3-chlorophenyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-methyl benzal)hydrazone **Vd**, 6-[4-(4-chlorophenyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-methyl benzal)hydrazone **Ve**, 6-[4-(pyridyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-methyl benzal)hydrazone **Vf** derivatives showed moderate analgesic activity. **Vh** and **Vi** bearing methoxy substituent on benzal hydrazone ring exhibited no inhibition against *p*-benzoquinone-induced writhings in mice. However, the mechanism underlying their antinociceptive activity remains to some degree unknown. Thus further studies are essential to ascertain the mechanism involved in the analgesic properties of the ring system.

Table 2
Analgesic activity of 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal)hydrazone **V** derivatives against *p*-benzoquinone-induced writhings in mice.

Test samples	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration
Control		42.7 ± 4.92		0/6
Va	100	20.3 ± 1.98	**52.5*****	0/6
Vb	100	21.5 ± 1.85	**49.6*****	0/6
Vc	100	19.4 ± 2.13	**54.6*****	0/6
Vd	100	31.0 ± 2.25	27.4	0/6
Ve	100	33.5 ± 4.02	21.5	0/6
Vf	100	26.1 ± 3.09	**38.9***	0/6
Vg	100	35.7 ± 2.41	16.4	0/6
Vh	100	47.4 ± 4.16	–	0/6
Vi	100	51.2 ± 3.98	–	0/6
ASA	100	21.9 ± 1.92	**48.7*****	5/6

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significant from the control value.

The anti-inflammatory activity evaluation was carried out using carrageenin-induced paw oedema assay, and compounds **Va**, **Vb**, **Vc** exhibited good anti-inflammatory activity. Anti-inflammatory activity of 6-[4-(3-chlorophenyl)piperazine]-3(2*H*)-pyridazinone-2-acetyl-2-benzal hydrazone **Va** has been found slight superior to that of indomethacin although it was tested at a dosage much higher than indomethacin. In light of these results, one that can say the *p*-substitution of benzal hydrazone aromatic ring with methoxy or methyl groups decreases either analgesic activity or anti-inflammatory activity.

We also screened all compounds for ulcerogenic adverse effect at 200 mg/kg dose level. After microscopic elimination, no ulceration risk was seen in compounds **Va**–**i**. It is well known that most of anti-inflammatory drugs provide an ulcerogenic activity. In the present experiment ASA and indomethacin used as reference showed marked ulcerogenic effect. Compounds **Va**, **Vb**, **Vc** possessed anti-inflammatory activity as well as indomethacin and more potent analgesic activity than ASA and did not induce any gastric lesions or death in the observation period. Since our findings are preliminary results; further studied need to be carried out to investigate the other specifications such as in vitro assays, toxicological studies or side effect-activity profiles of these compounds.

5. Experimental

All chemical melting points of the compounds were determined on Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the values given are uncorrected.

The IR spectra of the compounds were recorded on a Bruker Vector 22 IR Spectrophotometer (Bruker Analytische Messtechnik, Karlrure, Germany). The ^{1}H NMR of the compounds spectra were recorded on a Bruker 400 MHz-NMR Spectrometer (Rheinstetten, Karlrure, Germany) using tetramethylsilane as an internal standard. All the chemical shifts were recorded as δ (ppm). Elemental analyses were performed with Leco-932 (C, H, N, S, O-Elemental analyzer, St. Joseph, USA) at Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara, Turkey) and within ±0.4% of the theoretical values.

5.1. General procedure for the synthesis of compounds

5.1.1. Synthesis of 6-substituted-3(2H)-pyridazinone derivatives *IIa–i*

A solution of 0.05 mol of a 6-substituted-3-chloropyridazinone **I** derivative in 30 ml glacial acetic acid was refluxed for 6 h. The acetic acid was removed under reduced pressure, the residue dissolved in water and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by recrystallization from ethanol.

5.1.2. Synthesis of ethyl 6-substituted-3(2H)-pyridazinone-2-ylacetate derivatives *IIIa–I*

A mixture of required 6-substitited-3(2*H*)pyridazinones **II** (0.01 mol), ethyl bromoacetate (0.02 mol) and potassium carbonate (0.02 mol) in acetone (40 ml) was refluxed overnight. After the mixture was cooled, the organic salts were filtered off, the solvent evaporated, and the residue purified by recrystallization with appropriate alcohol to give the esters.

Table 3
Antiiflammatory activity of 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal)hydrazone **V** derivatives against carrageenan-induced paw edema in mice.

Test samples	Dose (mg/kg)	Swelling thickness ($\times 10^{-2}$mm) ± SEM (inhibition %)			
		90 min	180 min	270 min	360 min
Control		49.1 ± 4.16	56.4 ± 4.93	64.9 ± 5.02	73.5 ± 5.27
Va	100	37.6 ± 2.50 (23.4)	39.3 ± 2.82 **(30.3)***	40.5 ± 3.14 **(37.6)****	43.3 ± 3.19 **(41.0)*****
Vb	100	40.8 ± 2.94 (16.9)	42.7 ± 3.03 (24.2)	45.6 ± 3.22 **(32.7)****	49.2 ± 3.17 **(33.1)****
Vc	100	39.6 ± 2.51 (19.3)	41.2 ± 2.64 (26.9)	44.8 ± 2.16 **(30.9)***	49.6 ± 3.20 **(32.5)****
Vd	100	49.6 ± 3.02	57.3 ± 3.26	61.2 ± 3.91 (5.7)	69.8 ± 3.24 (5.0)
Ve	100	45.2 ± 2.83 (7.9)	49.6 ± 2.53 (12.1)	57.5 ± 3.06 (11.4)	59.9 ± 3.73 (18.5)
Vf	100	41.7 ± 2.64 (15.1)	45.8 ± 2.91 (18.8)	49.3 ± 3.02 (24.0)	51.1 ± 3.16 **(30.5)***
Vg	100	45.1 ± 2.64 (8.1)	49.8 ± 3.02 (11.7)	54.1 ± 3.19 (16.6)	60.2 ± 4.01 (18.1)
Vh	100	50.2 ± 4.11	59.4 ± 4.72	65.8 ± 4.81	70.9 ± 3.92 (3.5)
Vi	100	47.6 ± 3.24 (3.1)	54.2 ± 2.98 (3.9)	67.9 ± 3.15	74.1 ± 3.27
Indomethacin	10	32.2 ± 3.14 **(34.4)***	37.9 ± 3.02 **(32.8)***	40.6 ± 2.94 **(37.4)****	43.8 ± 2.71 **(40.4)*****

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significant from the control value.

*5.1.3. Synthesis of 6-substituted-3(2*H*)-pyridazinone-2-yl acetohy drazide derivatives* ***IVa–I***

To methanolic solution of ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate derivatives **III** (25 ml, 0.01 mol) was added hydrazine hydrate (99%) (3 ml) and stirred for 3 h in the room temperature. The precipitate obtained was filtered off, washed with water, dried and recrystallized from ethanol.

*5.1.4. Synthesis of 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted benzal)hydrazone* ***V*** *derivative*

'Mixture of 6-substituted-3(2*H*)-pyridazinone-2-yl acetohydrazide derivatives **IV** (0.01 mol) and appropriate benzaldehyde (0.01 mol) was refluxed in ethanol (15 ml) for 6 h. Then the mixture was poured into ice-water. The precipitate formed was recrystallized from ethanol.

5.1.4.1. Compound ***Va****.* IR (KBr) ν_{max} (cm^{-1}): 3185 N–H, 1681 C=O hydrazone, 1652 C=O lactam, 1595–1515 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.20–3.27 (m, 4H, piperazine b + b′), 3.28–3.34 (m, 4H, piperazine a + a′), 4.82 and 5.03 (2H, s, s, CH_2), 6.88–6.91 (d, 1H, pyridazinone H_4), 6.96–7.70 (m, 10H, phenyl protons + pyridazinone H_5), 7.98 and 8.18 (1H, s, s, N=CH), 11.64 (1H, s, NH). Anal. Calc. for $C_{23}H_{23}ClN_6O_2$: C: 61.26, H: 5.14, N: 18.64. Found: C: 61.38, H: 5.21, N: 18.83.

5.1.4.2. Compound ***Vb****.* IR (KBr) ν_{max} (cm^{-1}): 3182 N–H, 1683 C=O hydrazone, 1653 C=O lactam, 1590–1510 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.26–3.33 (m, 4H, piperazine b + b′), 3.34–3.40 (m, 4H, piperazine a + a′), 4.63 and 5.01(2H, s, s, CH_2), 6.78–6.86 (d, 1H, pyridazinone H_4), 6.91–7.70 (m, 10H, phenyl protons+ + pyridazinone H_5), 7.99 and 8.18 (1H, s, s, N=CH), 11.61 (1H, s, NH). Anal. Calc. for $C_{23}H_{23}ClN_6O_2$: C: 61.26, H: 5.14, N: 18.64. Found: C: 61.46, H: 5.10, N: 18.43.

5.1.4.3. Compound ***Vc****.* IR (KBr) ν_{max} (cm^{-1}): 3185 N–H, 1682 C=O hydrazone, 1658 C=O lactam, 1593–1512 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.25–3.33 (m, 4H, piperazine b + b′), 3.34–3.40 (m, 4H, piperazine a + a′), 4.68 and 5.02 (2H, s, s, CH_2), 6.78–6.80 (d, 1H, pyridazinone H_4), 6.96–7.70 (m, 10H, pyridyl protons + pyridazinone H_5), 7.98 and 8.17 (1H, s, s, N=CH), 11.60 (1H, s, NH), Anal. Calc. for $C_{22}H_{23}N_7O_2$: C: 63.30, H: 5.55, N: 23.49. Found: C: 63.42, H: 5.62, N: 23.83.

5.1.4.4. Compound ***Vd****.* IR (KBr) ν_{max} (cm^{-1}): 3189 N–H, 1678 C=O hydrazone, 1661 C=O lactam, 1590–1510 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.28 (s, 3H, CH_3), 3.18–3.35 (m, 4H, piperazine b + b′), 3.42–3.49 (m, 4H, piperazine a + a′), 4.60 and 5.00 (2H, s, s, CH_2), 6.85–6.91 (d, 1H, pyridazinone H_4), 6.97–7.63 (m, 9H, phenyl protons + pyridazinone H_5), 7.97 and 8.12 (1H, s, s, N=CH), 11.57 (1H, s, NH), Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C: 62.00, H: 5.42, N: 18.08. Found: C: 62.13, H: 5.51, N: 18.33.

5.1.4.5. Compound ***Ve****.* IR (KBr) ν_{max} (cm^{-1}): 3186 N–H, 1680 C=O hydrazone, 1660 C=O lactam, 1592–1509 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.29 (s, 3H, CH_3), 3.23–3.36 (m, 4H, piperazine b + b′), 3.41–3.47 (m, 4H, piperazine a + a′), 4.61 and 5.03 (2H, s, s, CH_2), 6.76–6.89 (d, 1H, pyridazinone H_4), 6.96–7.62 (m, 9H, phenyl protons + pyridazinone H_5), 7.93 and 8.13 (1H, s, s, N=CH), 11.56 (1H, s, NH), Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C: 62.00, H: 5.42, N: 18.08. Found: C: 61.96, H: 5.30, N: 18.24.

5.1.4.6. Compound ***Ve****.* IR (KBr) ν_{max} (cm^{-1}): 3182 N–H, 1677 C=O hydrazone, 1658 C=O lactam, 1589–1509 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.30 (s, 3H, CH_3), 3.24–3.35 (m, 4H, piperazine b + b′), 3.42–3.48 (m, 4H, piperazine a + a′), 4.61 and 5.03 (2H, s, s, CH_2), 6.76–6.89 (d, 1H, pyridazinone H_4), 6.96–7.62 (m, 9H, phenyl protons + pyridazinone H_5), 7.93 and 8.13 (1H, s, s, N=CH), 11.58 (1H, s, NH), Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C: 62.00, H: 5.42, N: 18.08. Found: C: 61.96, H: 5.30, N: 18.24.

5.1.4.7. Compound ***Vf****.* IR (KBr) ν_{max} (cm^{-1}): 3181 N–H, 1679 C=O hydrazone, 1661 C=O lactam, 1590–1508 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 2.30 (s, 3H, CH_3), 3.24–3.35 (m, 4H, piperazine b + b′), 3.41–3.47 (m, 4H, piperazine a + a′), 4.61 and 5.01 (2H, s, s, CH_2), 6.77–6.87 (d, 1H, pyridazinone H_4), 6.93–7.62 (m, 9H, pyridyl protons + pyridazinone H_5), 7.95 and 8.14 (1H, s, s, N=CH), 11.61 (1H, s, NH), Anal. Calc. for $C_{23}H_{25}N_7O_2$: C: 64.02, H: 5.84, N: 22.72. Found: C: 64.21, H: 5.60, N: 22.31.

5.1.4.8. Compound ***Vg****.* IR (KBr) ν_{max} (cm^{-1}): 3180 N–H, 1679 C=O hydrazone, 1659 C=O lactam, 1591–1510 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.18–3.35 (m, 4H, piperazine b + b′), 3.36–3.43 (m, 4H, piperazine a + a′), 3.75 (s, 3H, OCH_3), 4.59 and 4.99 (2H, s, s, CH_2), 6.86–6.94 (d, 1H, pyridazinone H_4), 6.97–7.62 (m, 9H, phenyl protons + pyridazinone H_5), 7.91 and 8.10 (1H, s, s, N=CH), 11.50 (1H, s, NH), Anal. Calc. for $C_{24}H_{25}ClN_6O_3$: C: 59.94, H: 5.24, N: 17.47. Found: C: 59.63, H: 5.43, N: 17.13.

5.1.4.9. Compound ***Vh****.* IR (KBr) ν_{max} (cm^{-1}): 3181 N–H, 1680 C=O hydrazone, 1660 C=O lactam, 1590–1510 C=N, ^{1}H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.24–3.34 (m, 4H, piperazine b + b′), 3.38–3.48 (m, 4H, piperazine a + a′), 3.76 (s, 3H, OCH_3), 4.60 and 4.99 (2H, s, s, CH_2), 6.76–6.90 (d, 1H, pyridazinone H_4), 6.95–7.63 (m, 9H, phenyl protons + pyridazinone H_5), 7.92 and 8.11 (1H, s, s, N=CH), 11.51 (1H, s, NH), Anal. Calc. for $C_{24}H_{25}ClN_6O_3$: C: 59.94, H: 5.24, N: 17.47. Found: C: 59.47, H: 5.18, N: 17.24.

5.1.4.10. Compound ***Vf***. IR (KBr) ν_{max} (cm^{-1}): 3182 N–H, 1682 C=O hydrazone, 1662 C=O lactam, 1592–1510 C=N, ^1H NMR (300 MHz) (DMSO d_6) δ (ppm): 3.23–3.34 (m, 4H, piperazine b + b′), 3.40–3.49 (m, 4H, piperazine a + a′), 3.75 (s, 3H, OCH_3), 4.60 and 5.00 (2H, s, s, CH_2), 6.76–6.89 (d, 1H, pyridazinone H_4), 6.93–7.62 (m, 9H, pyridyl protons + pyridazinone H_5), 7.91 and 8.10 (1H, s, s, N=CH), 11.49 (1H, s, NH), Anal. Calc. for $C_{23}H_{25}N_7O_3$: C: 61.73, H: 5.63, N: 21.91. Found: C: 61.90, H: 5.42, N: 21.61.

5.2. Analgesic and anti-inflammatory activity

5.2.1. Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for two days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn one day before the experiment, but allowed free access of water. Six animals at least were used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

5.2.2. Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethylcellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indometacin (CAS 53-86-1) (10 mg/kg) or acetylsalicylic acid (CAS 50-78-2; ASA) (100 mg/kg) in 0.5% CMC was used as reference drug.

5.2.3. Analgesic and anti-inflammatory activity tests

5.2.3.1. p-*Benzoquinone-induced abdominal constriction test in mice [25].* 60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent average of the total number of writhings observed. The antinociceptive activity was expressed as writhing percentage of that of control′s. 100 mg/kg acetylsalicylic acid (ASA) was used as the reference.

5.2.3.2. Carrageenan-induced hind paw edema [26]. The method of Kasahara et al. (1985) was used with modifications in measuring periods. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. 60 min after the oral administration of test sample or dosing vehicle each mouse was injected with freshly prepared (0.5 mg/25 μl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 nmol/l NaCl) into subplantar tissue of the right hind paw. As the control, 25 μl saline solutions were injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as reference drug.

5.2.4. Acute toxicity

Animals employed in the carrageen-induced paw edema experiment were observed during 24 h and mortality was recorded, if happens, for each group at the end of observation period.

5.2.5. Gastric ulcerogenic effect

After the analgesic activity experiment mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the great curvature and examined under dissecting microscope for lesions or bleedings.

5.2.6. Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Students–Newman–Keels post-hoc tests. $p < 0.05$ was considered to be significant [$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$].

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