

Synthesis and Analgesic, Antiinflammatory and Antimicrobial Evaluation of 6-Substituted-3(2H)-Pyridazinone-2-acetyl-2- (Substituted Benzal) Hydrazone Derivatives

Zeynep ÖZDEMİR*, Mehtap GÖKÇE**, Arzu KARAKURT*

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Summary

In present study a series of new 6-substituted-3(2H)-pyridazinone-2-acetyl-2- (substituted benzal) hydrazone derivatives were synthesized and evaluated for analgesic, and anti-inflammatory and antimicrobial activities. The structures of these new pyridazinone derivatives were confirmed by their IR, ¹H-NMR spectra and elementary analysis. Analgesic activity of new pyridazinone derivatives have been tested by the phenylbenzoquinone-induced writhing test (PBQ test), and anti-inflammatory activity have been evaluated the carragenan-induced paw edema method. A significant dependence of the anti-inflammatory and analgesic effects on the substituents has been observed. The pharmacological study of these compounds confirms that modification of the atom or chemical group at phenyl ring of benzalhydrazone moiety influences analgesic and anti-inflammatory activities. Synthesized compounds have been shown moderate antimicrobial activities.

Key Words: 6-Substituted-3 (2H) -pyridazinones, Benzaldehyde Hydrazones, Analgesic activity, Anti-inflammatory activity.

6-Substitüie-3(2H) -pidazinon -2-asetil-2- (süstitüie benzal) hidrazon Türevlerinin Sentezi ve Analjezik, Antiinflamatuvar ve Antimikrobiyal Aktivitelerinin Değerlendirilmesi

Özet

Sunulan bu çalışmada 6-süstitüie-3(2H)-pidazinon-2-asetil-2-(süstitüie benzal) hidrazon türevlerinin yeni bir serisi sentezlenmiş ve analjezik, antiinflamatuvar ve antimikrobiyal aktiviteleri değerlendirilmiştir. Yeni pidazinon türevlerinin yapısı IR, ¹H-NMR spektrumları ve elementel analiz ile doğrulanmıştır. Yeni pidazinon türevlerinin analjezik aktivitesi phenylbenzokinon ile indüklenen kıranma testi, antiinflamatuvar aktivitesi karragenin ile indüklenen pençe ödemi testi ile değerlendirilmiştir. Antiinflamatuvar ve analjezik aktivitede süstitüientlere belirgin bir bağımlılık gözlenmiştir. Bileşiklerin farmakolojik çalışmaları benzalhidrazon yapısının fenil halkası üzerindeki atom veya kimyasal grupların modifikasyonunun analjezik ve antiinflamatuvar aktiviteyi etkilediğini doğrulamıştır. Sentezlenen bileşikler orta düzeyde antimikrobiyal aktiviteler göstermişlerdir.

Anahtar Kelimeler: 6-Süstitüie-3(2H)-pidazinonlar, Benzaldehit Hidrazonlar, Analjezik Aktivite, Anti-enflamatuvar aktivite

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* İnönü University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 44280, Malatya, Turkey

** Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06330, Ankara, Turkey

o Corresponding Author E-mail: mgokce99@gmail.com,

INTRODUCTION

A number of hydrazide-hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial-antifungal (1), anticonvulsant (2), antiinflammatory (3), antimalarial (4), analgesic (5,6), antiplatelets (7), antituberculosis (8) and anticancer activities (9). Aroylhydrazide-hydrazones containing hetero-ring such as pyridine (3,10), indole (11), 1,2,4-oxadiazole (5), 1,2,3-triazole (6) and imidazo [2,1-b] thiadiazole ring (8) have attracted special attention. A few of pyrazole carbohydrazone derivatives have also been reported (12,13).

The diversity, efficiency and rapid access to small and highly functionalized organic molecules make this approach of central current interest in the construction of combinatorial libraries and optimization in drug discovery process. The pyridazinone nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs. Some of the present day drugs such as emorfazone (14) (analgesic), pibobendan (15) (positive inotropic, vasodilator), levosimendan (16) (calcium sensitizer), imazodan (17) (cardiotonic), zardaverine (18) (phosphodiesterase inhibitor), amrinone (19) (positive inotropic agent), milrinone (20) (vasodilator), are the best examples for potent molecules possessing pyridazinone nucleus.

Furthermore the ability of nonsteroidal anti-inflammatory drugs (NSAIDs) to modulate the pain, inflammation and fever made them one of the most used therapeutical classes in the world. The utility of nonsteroidal anti-inflammatory drugs in the treatment of inflammation and pain is often limited by gastrointestinal liabilities including ulceration and bleeding. 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. Due to favorable presence a pyridazinone moiety in known active structures, pyridazinone

derivatives provoked a special interest in the search for new analgesic, anti-inflammatory (3, 5, 6, 21-28) and antimicrobial (1,8, 29-31) agents. In view of the above mentioned findings and as continuation of our effort (32-37) to identify new candidates that may be of value in designing new, potent, selective and less toxic analgesic, anti-inflammatory antimicrobial agents, we report herein the synthesis of some new 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted benzal) hydrazone V derivatives.

MATERIAL AND METHODS

Apparatus

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, Karlsruhe, Germany). The ¹H-NMR of the compounds spectra were recorded on a Bruker Avance 300 MHz Ultrashield™ NMR Spectrometer using tetramethylsilane as an internal standard at İnönü University Scientific and Technical Research Laboratory Center. All the chemical shifts were recorded as δ (ppm). High resolution mass spectra data (HRMS) were collected inhouse using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in ESI (s) method, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory.

Chemistry

The fine chemicals and all solvents used in this study were purchased locally from E. Merck (Darmstadt, F. R. Germany) and Aldrich Chemical Co. (Steinheim, Germany). 3-Chloro-6-(4-benzylpiperidine) pyridazine I was carried out by the reaction 3,6-dichloropyridazine with 4-benzylpiperidine (38-39).

Synthesis of 6- (4-benzylpiperidine)-3(2H)-pyridazinone derivatives II (32)

A solution of 0.05 mol of a 3-chloro-6-(4-benzylpiperidine) pyridazine 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.

Synthesis of ethyl 6- (4-benzylpiperidine)-3(2H)-pyridazinone-2-ylacetate derivatives III (33)

A mixture of required 6-(4-benzylpiperidine)-3(2H)-pyridazinone II (0.01 mol), ethyl chloroacetate (0.02 mol) and potassium carbonate (0.02 mol) in 40 mL acetone were refluxed overnight. After the mixture was cooled, the resulting organic salts were filtered off, the filtrate was evaporated, and the residue purified by recrystallization with appropriate alcohol to give the esters.

Synthesis of 6- (4-Benzylpiperidine)-Substituted-3(2H)-pyridazinone-2-yl acetohydrazide derivatives IV

To methanolic solution of ethyl 6-(4-Benzylpiperidine)-3 (2H)-pyridazinone-2-ylacetate derivatives III (0.01 mol) were added 3 mL hydrazine hydrate (99 %) and stirred for 3 h in the room temperature. The resulting crude precipitate was filtered off, purified by repeated washing with cold water, and dried in vacuo.

General procedure for synthesis of 6-(4-Benzylpiperidine)-3(2H)-pyridazinone-2-acetyl-2-(substituted/ nonsubstituted benzaldehyde) hydrazone derivatives V

Mixture of 6- (4-benzylpiperidine) -3 (2H) -pyridazinone-2-yl-acetohydrazide derivatives IV (0.01 mol) and appropriate non substituted/substituted benzaldehyde (0.01 mol) were refluxed in 15 mL ethanol for 6 h. Then the mixture was poured into ice-water. The precipitate formed was recrystallized from ethanol.

Pharmacological Activity

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.

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.

p-Benzoquinone-induced abdominal constriction test in mice (40)

60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10g 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.

Carrageenan-induced hind paw edema (41)

The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers. 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. Indometacin (10 mg/kg) was used as reference drug.

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.

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.

Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error (\pm 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 [* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$].

Antibacterial and Antifungal Activity

Material

The following bacteria were used for antibacterial study: Standard strains of *E.coli* ATCC 25922, *E.coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212. The following yeast-like fungi were used for antifungal study; *Candida albicans* ATCC 10231 and *Candida krusei* ATCC 6258.

Inoculation suspensions

The microorganism suspensions used for inoculation were prepared at 10^6 cfu/ml concentration by

diluting of the fresh cultures at McFarland 0.5 density (10^8 cfu/ml). It was known that there were 5×10^4 cfu/ml microorganisms in each well after inoculation.

Medium

Mueller Hinton Broth (Oxoid) liquid nutrient medium was used for diluting of microorganism suspension and two fold-dilution of the compounds. Sabouraud liquid medium (Oxoid) was used for yeast like fungi for the same purpose.

Equipment

Falcon^R microplates which have 96 wells were used for microdilution method. Brinkmann transferpette was used for two fold-dilution of compounds in the wells.

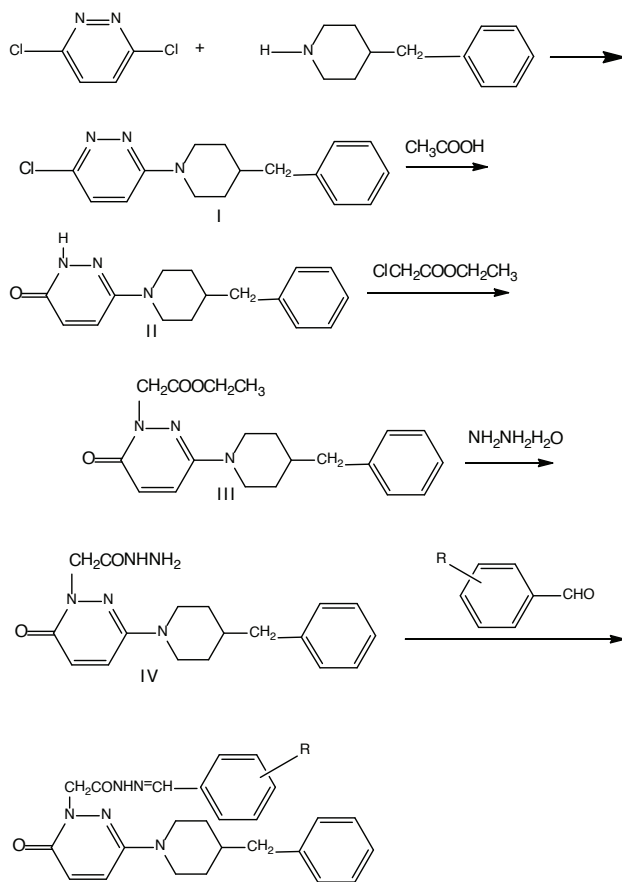
Method

Microdilution method was employed for antibacterial and antifungal activity tests (42). The synthesized compound and the standarts ampicillin trihydrate and fluconazole were dissolved in DMSO at 1000 mg/ml concentration at the beginning. The solution of each compounds at 500-3.9 mg/ml were prepared in the wells by diluting with the mediums. Suspension of the microorganisms at 10^6 cfu/ml concentration were inoculated to the two fold-diluted solution of the compounds, consequently the microorganism concentration in each well was approximately 5×10^4 cfu/ml. DMSO-microorganisms mixture, the pure microorganisms, and pure media were used as control wells.

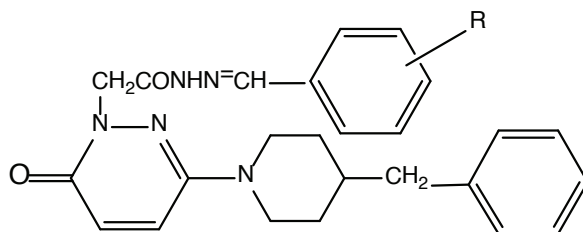
Microplates were covered and incubated at 36°C for 24-48 hours. Wet cotton-wool was placed in the incubation chamber, because it should be kept sufficiently to avoid evaporation. After this period of time, evaluation of the wells was performed. The concentration of the compounds in the wells where no growth was assessed as the minimum inhibitory concentration (MIC) of the compounds. There was no inhibitory activity in the wells containing only DMSO. The microbial growth occurred, and the medium were not contaminated during the tests. The MIC values of 6-substituted-3(2H)-pyridazinone-2-acetyl-2- (substituted benzal) hydrazone V derivatives were given in Table 4.

RESULTS AND DISCUSSION

New 6-(4-benzylpiperidine)-3(2*H*)-pyridazinone-2-acetyl-2-(substituted benzal) hydrazone V derivatives were synthesized according to Scheme 1. Initially, nucleophilic displacement reaction of commercial 3,6-dichloropyridazine with arylpiperidines in ethanol afforded 3-chloro-6-(4-benzylpiperidine)-pyridazines I. The physical and spectral properties of 3-chloro-6-(4-benzylpiperidine) pyridazine I were accordance with the literature (38,39). Therefore we carried out the next steps of the reaction without any further analysis. Hydrolysis of 3-chloro-6-(4-benzylpiperidine) pyridazine I were carried out upon heating in glacial acetic acid to afford 6-(benzylpiperidine)-3(2*H*)-pyridazinone II derivatives (32). The formation of these compounds were confirmed by IR spectra of a C=O signal at about 1660 cm^{-1} . Ethyl 6-(benzylpiperidine)-3(2*H*)-pyridazinone-2-ylacetate III derivatives were obtained by the reaction of II with ethyl 2-chloroacetate in the presence of K_2CO_3 in acetone (33). 6-(Benzylpiperidine)-3(2*H*)-pyridazinone-2-yl acetohydrazide derivatives IV were synthesized by the condensation reaction of ethyl 6-(benzylpiperidine)-3(2*H*)-pyridazinone-2-ylacetate III derivatives with hydrazine hydrate (99%). All of the 6-(Benzylpiperidine)-3(2*H*)-pyridazinone-2-acetyl-2-(substituted/nonsubstitutedbenzal) hydrazone V derivatives were reported first time in this study. Synthesized V derivatives have been given Table 1. The melting point, yield and molecular formula of all compounds are reported Table 1.



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

Table 1. The physical data, yield and molecular formula of 6-substituted-3(2*H*)-pyridazinone-2- acetyl-2-(*p*-substituted benzal) hydrazone V derivatives.

Com.	R	Mp (°C)	Yield (%)	Molecular Formula (MW)
Va	H	170-172	43	$C_{25}H_{27}N_5O_2$ (Calc.: 429.2254; Found: 429.2043)
Vb	4-Br	195-197	74	$C_{25}H_{26}BrN_5O_4$ (Calc.: 507.1341; Found: 507.1132)
Vc	2-Cl	233-235	76	$C_{25}H_{26}ClN_5O_4$ (Calc.: 463.1896; Found: 463.1987)
Vd	4-Cl	119-121	56	$C_{25}H_{26}ClN_5O_4$ (Calc.: 463.1896; Found: 463.1983)
Ve	2-F	182-184	61	$C_{25}H_{26}FN_5O_4$ (Calc.: 447.2150; Found: 447.2063)
Vf	4-F	210-312	46	$C_{25}H_{26}FN_5O_4$ (Calc.: 447.2150; Found: 447.2059)
Vg	4-CH ₃	202-204	67	$C_{26}H_{29}N_5O_2$ (Calc.: 443.2354; Found: 443.2446)
Vh	3-OCH ₃	201-203	71	$C_{26}H_{29}N_5O_3$ (Calc.: 459.2354; Found: 459.2432)
Vi	4-OCH ₃	162-164	28	$C_{26}H_{29}N_5O_3$ (Calc.: 459.2354; Found: 459.2423)
Vj	4-N(CH ₃) ₂	204-206	34	$C_{27}H_{32}N_6O_2$ (Calc.: 472.2645; Found: 472.2533)
Vk	4-NO ₂	223-225	32	$C_{25}H_{26}N_6O_4$ Calc.: 474.2063; Found: 474.2254)

Table 2. Spectral data of 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(p-substituted benzal) hydrazone V derivatives. (see Table 1 for structural formula).

Comp	IR (KBr) cm^{-1}			^1H NMR ($\text{DMSO}-d_6$) ppm (δ)
	C=O (Chain)	C=O (Pyridazinone ring)	C=N	
Va	1780	1650	1590	1.08-1.59 (1H, m, piperidin proton), 2.40-2.52 (4H, m, piperidin protons), 3.53 (s, 2H, benzyl CH_2), 3.72-3.82 (4H piperidin protons), 4.37 and 4.65 (2H, s, s, $\text{CH}_2\text{-CO-}$), 6.76-6.84 (1H, d, pyridazinone H_4), 7.10-7.62 (m, 11H, phenyl protons+pyridazinone H_5), 9.70-9.95 (1H, s, s, N=CH), 10.40 (1H, s, CO-NH-N).
Vb	1782	1655	1595	1.08-1.60 (1H, m, piperidin proton), 2.49-2.79 (4H piperidin protons), 3.51 (s, 2H, benzyl CH_2), 3.74-3.86 (4H piperidin protons), 4.40 and 4.65 (2H, s, s, CH_2), 6.78-6.89 (1H, d, pyridazinone H_4), 7.16-7.67 (m, 10H, phenyl protons+pyridazinone H_5), 9.77-9.96 (1H, s, s, N=CH), 10.42 (1H, s, CO-NH-N).
Vc	1781	1665	1597	1.10-1.62 (1H, m, piperidin proton), 2.44-2.75 (4H piperidin protons), 3.53 (s, 2H, benzyl CH_2), 3.76-3.87 (4H piperidin protons), 4.42 and 4.67 (2H, s, s, CH_2), 6.79-6.89 (1H, d, pyridazinone H_4), 7.26-7.85 (m, 10H, phenyl protons + pyridazinone H_5), 9.65-9.96 (1H, s, s, N=CH), 10.43 (1H, s, CO-NH-N).
Vd	1780	1660	1598	1.09-1.60 (1H, m, piperidin proton), 2.49-2.73 (4H piperidin protons), 3.32-3.55 (4H piperidin protons), 3.56 (s, 2H, benzyl CH_2), 4.43 and 4.69 (2H, s, s, CH_2), 6.76-6.87 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.47 (1H, s, CO-NH-N).
Ve	1781	1665	1599	1.10-1.60 (1H, m, piperidin proton), 2.47-2.73 (4H, m, piperidin protons), 3.40-3.72 (4H, m, piperidin protons), 3.53 (s, 2H, benzyl CH_2), 4.38 and 4.64 (2H, s, s, CH_2), 6.70-6.86 (1H, d, pyridazinone H_4), 7.28-7.89 (m, 10H, phenyl protons + pyridazinone H_5), 9.67-9.96 (1H, s, s, N=CH), 10.45 (1H, s, CO-NH-N).
Vf	1782	1662	1570	1.13-1.63 (1H, m, piperidin proton), 2.47-2.73 (4H piperidin protons), 3.42-3.75 (4H piperidin protons), 3.54 (s, 2H, benzyl CH_2), 4.39 and 4.66 (2H, s, s, CH_2), 6.71-6.87 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.45 (1H, s, CO-NH-N).
Vg	1781	1664	1580	1.12-1.59 (1H, m, piperidin proton), 2.36-2.38 (3H, s, CH_3 protons), 2.47-2.73 (4H, m, piperidin protons), 3.42-3.75 (4H piperidin protons), 3.55 (s, 2H, benzyl CH_2), 4.39 and 4.66 (2H, s, s, CH_2), 6.70-6.86 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.44 (1H, s, CO-NH-N).
Vh	1781	1663	1585	1.10-1.60 (1H, m, piperidin proton), 2.46-2.72 (4H, piperidin protons), 3.42-3.75 (4H piperidin protons), 3.54 (s, 2H, benzyl CH_2), 3.79 (3H, s, OCH_3), 4.39 and 4.66 (2H, s, s, CH_2), 6.71-6.87 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.44 (1H, s, CO-NH-N).
Vi	1780	1665	1590	1.12-1.59 (1H, m, piperidin proton), 2.47-2.73 (4H piperidin protons), 3.42-3.75 (4H piperidin protons), 3.54 (s, 2H, benzyl CH_2), 3.77 (3H, s, OCH_3), 4.39 and 4.66 (2H, s, s, CH_2), 6.71-6.87 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.67-9.96 (1H, s, s, N=CH), 10.43 (1H, s, CO-NH-N).
Vj	1785	1667	1592	1.11-1.61 (1H, m, piperidin proton), 2.47-2.73 (4H, m, piperidin protons), 2.97 (6H, s, -N (CH_3) ₂), 3.42-3.75 (4H piperidin protons), 3.54 (s, 2H, benzyl CH_2), 4.39 and 4.66 (2H, s, s, CH_2), 6.70-6.88 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.46 (1H, s, CO-NH-N).
Vk	1785	1667	1592	1.13-1.63 (1H, m, piperidin proton), 2.47-2.73 (4H, m, piperidin protons), 3.44-3.79 (4H piperidin protons), 3.54 (s, 2H, benzyl CH_2), 4.39 and 4.66 (2H, s, s, CH_2), 6.71-6.87 (1H, d, pyridazinone H_4), 7.28-7.99 (m, 10H, phenyl protons + pyridazinone H_5), 9.68-9.97 (1H, s, s, N=CH), 10.47 (1H, s, CO-NH-N).

Analgesic and Antiinflammatory Activity

Synthesized 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted benzal) hydrazone V derivatives were tested for analgesic activity by the phenylbenzoquinone-induced writhing test (40) and for anti-inflammatory activity using the carrageenan-induced paw edema method (41). The animals have

been shown no observable effect to the test compounds. The animals tolerated the tests well and no animals were died duration of the experiments. All the pyridazinone derivatives have also been evaluated for acute toxicity and gastric ulcerogenic effect tests. The obtained results are reported in Table 3.

Table 3. Effect of 6-Substituted-3(2H)-pyridazinone-2-acetyl-3-(substituted benzal) hydrazone V derivatives against carrageenan-induced paw edema and p- benzoquinone-induced writhings tests in mice

Compound	Dose, mg/kg, Per os	Anti-inflammatory Activity Thickness of edema \pm SEM (Inhibition %)				Analgesic activity Number of stretching (Inhibition %)	Gastric Ulcerogenic effect
		90 min	180 min	270 min	360 min		
Control		45.0 \pm 4.5	51.8 \pm 4.8	60.0 \pm 4.7	68.0 \pm 5.0	44.3 \pm 3.9	0/6
Va	100	45.0 \pm 4.4 (4.8)	50.0 \pm 4.8 (3.5)	53.7 \pm 4.9 (10.5)	49.3 \pm 2.6 (27.5) **	29.5 \pm 2.5 (33.4) ***	0/6
Vb	100	42.2 \pm 3.5 (6.2)	46.8 \pm 3.7 (9.7)	51.8 \pm 3.4 (13.7)	57.0 \pm 3.9 (16.2)	31.7 \pm 2.2 (28.4) **	0/6
Vc	100	41.3 \pm 3.0 (8.2)	45.7 \pm 3.0 (11.7)	43.3 \pm 3.9 (27.8) **	47.3 \pm 4.5 (30.4) **	29.3 \pm 2.2 (33.9) **	0/6
Vd	100	42.2 \pm 3.5 (6.2)	46.8 \pm 3.7 (9.7)	51.8 \pm 3.4 (13.7)	57.0 \pm 3.9 (16.2)	31.7 \pm 2.2 (28.4) **	0/6
Ve	100	34.5 \pm 3.0 (23.3)	38.3 \pm 2.9 (26.1)	41.2 \pm 2.5 (31.3) *	44.2 \pm 2.5 (35.0) **	13.8 \pm 1.5 (68.8) ***	0/6
Vf	100	33.5 \pm 3.2 (26.6)	37.8 \pm 2.7 (27.0)	41.8 \pm 1.7 (30.3) *	46.5 \pm 2.1 (31.6) **	29.3 \pm 2.8 (33.9) ***	0/6
Vg	100	41.8 \pm 3.8 (8.2)	46.8 \pm 3.9 (9.7)	52.3 \pm 4.2 (12.8)	56.7 \pm 3.4 (36.6) ***	16.3 \pm 1.3 (63.2) ***	0/6
Vh	100	42.3 \pm 3.5 (6.0)	47.5 \pm 4.0 (8.3)	52.2 \pm 3.7 (12.8)	55.7 \pm 3.4 (20.5)	29.8 \pm 3.2 (32.7) **	0/6
Vi	100	37.8 \pm 2.64 (4.8)	43.5 \pm 2.96 (5.0)	50.1 \pm 3.11 (10.1)	57.4 \pm 3.23 (6.5)	39.2 \pm 3.02 (19.0)	0/6
Vj	100	40.1 \pm 2.12	39.2 \pm 2.41 (14.4)	40.2 \pm 2.92 (27.8) *	42.3 \pm 3.05 (31.1) **	41.5 \pm 2.76 (14.2)	0/6
Vk	100	41.3 \pm 3.23 (11.6)	44.7 \pm 3.32 (18.4)	41.7 \pm 3.12 (24.5)	48.5 \pm 3.23 (23.5)	30.7 \pm 2.38 (31.5) **	0/6
Indomethacin	10	32.7 \pm 3.1 (27.3)	35.3 \pm 2.8 (31.9) *	37.8 \pm 2.4 (37.0) **	41.2 \pm 1.8 (39.4) ***		4/6
ASA	100					21.8 \pm 2.1 (50.8) ***	5/6

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

Table 4. *In vitro* antibacterial and antifungal activity of 6-Substituted-3(2*H*)-pyridazinone-2-acetyl-2-(substituted benzal) hydrazone V derivatives

Compound	A	B	C	D	E	F	G	H	I	J	K
Va	128	128	128	128	128	64	64	128	128	64	64
Vb	128	128	128	128	128	64	64	128	128	64	64
Vc	128	128	128	128	128	64	64	128	128	64	64
Vd	128	128	128	128	128	64	64	128	128	64	64
Ve	128	128	128	128	128	32	64	128	128	64	64
Vf	128	128	128	128	128	256	64	128	128	64	64
Vg	128	128	128	128	128	256	64	128	128	64	64
Vh	128	128	128	128	128	256	64	128	128	64	64
Vi	128	128	128	128	128	256	64	128	128	64	64
Vj	128	128	128	64	128	128	64	128	128	64	64
Vk	128	128	128	64	128	128	64	128	128	64	64
Ampicillin	2	-	>1024	-	-	0.5	-	0.5	0.5	-	-
Gentamicin	0.25	-	256	1	64	0.5	128	8	8	-	-
Ofloxacin	0.015	-	16	1	1	0.125	0.5	1	4	-	-
Rifampicin	16	-	256	32	128	0.004	2	0.5	4	-	-
Tetracyclin	0.5	-	256	8	128	0.25	8	8	16	-	-
Ceftriaxon	0.125	-	512	64	64	2	-	-	-	-	-
Meropenem	0.008	-	0.015	0.25	0.015	0.03	-	4	8	-	-
Eritromycin	-	-	-	-	-	0.25	16	1	0.25	-	-
Vancomycin	-	-	-	-	-	0.5	1	1	8	-	-
Ampicillin Sulbactam	-	16	-	-	-	-	-	-	-	-	-
Amoxicillin clavulonic acid	-	16	-	-	-	-	-	-	-	-	-
Fluconazol	-	-	-	-	-	-	-	-	-	0.0625	32
Amphotericin B	-	-	-	-	-	-	-	-	-	<0.03	0.5

A: *E.coli* ATCC 25922, B: *E.coli* ATCC 35218, C: *E.coli* isolat (ESBL), D: *Pseudomonas aeruginosa* ATCC 27853, E: *P.aeruginosa* isolat (Resistant to gentamycin), F: *Staphylococcus aureus* ATCC 29213, G: *S.aureus* isolat (Resistant to Methicillin), H: *Enterococcus faecalis* ATCC 29212, I: *E.faecalis* isolat (Resistant to Vancomycin), J: *Candida albicans* ATCC 10231, K: *C.krusei* ATCC 6258

As it seen Table 3; compound Ve was found to be more active than acetylsalicylic acid (ASA). Similarly, anti-inflammatory activity of Ve has been found close to that of indometacin in carrageenan-induced paw edema test. A moderate activity was generally shown by all remaining compounds, although they were tested at a dosage much higher than indometacin. In the light of these finding, one might be inclined to say that substituted fluorophenylpiperazine substituents on the pyridazinone ring could be critical for analgesic and anti-inflammatory activity. Furthermore, It was reported that fluorophenylpiperazine moiety on the amine part of these compounds have positive influences on their analgesic and anti-inflammatory activity (43-46). This also agreement with our previous study (32-37).

In addition, It is well known that most of anti-inflammatory drugs provide an ulcerogenic activity. In the present experiment ASA and indometacin used as reference showed marked ulcerogenic effect. Thus it appeared that IVc ve and Vg possessed anti-inflammatory activity as well as indometacin and more potent analgesic activity than ASA and did not induce any gastric lesions or death the observation period. Most of non-steroidal anti-inflammatory drugs are acidic and generally referred to as drugs similar to acetylsalicylic acid (ASA). On the other hand a few chemically basic compounds such as benzydamine HCl, tiaramide HCl and mepirizole which have pharmacological properties in common with ASA-like drugs. One of the most interesting characteristic of 6-Substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted benzal) hydrazone V derivatives 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.

Antimicrobial Activity

Standard strains of *E.coli* ATCC 25922, *E.coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 and *Candida krusei* ATCC 6258 and clinical isolates of these microorganisms that are known to be resistant

to various antimicrobial agents were included in the study. Strains were provided from Gazi University Faculty of Medicine Department of Medical Microbiology. Standard powders of ampicillin, gentamycin sulphate, ofloxacin, rifampicin, tetracyclin, ceftriaxon, meropenem, eritromycin, vancomycin, ampicillin/sulbactam, amoxicillin/clavulonic acid, fluconazole and amphotericin B were obtained from the manufacturers.

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