

Studies on Some 3-Oxo-5-benzylidene-6-methyl-(4H) - 2 - substituted pyridazines with Antinociceptive and Antiinflammatory Activities

Ayla BALKAN*^o, Fügen ÖZKANLI*, Oya ÜNSAL*, Zafer GÖREN**, Berna TERZİOĞLU**

Studies on Some 3-Oxo-5-benzylidene-6-methyl-(4H)-2-substituted pyridazines with Antinociceptive and Antiinflammatory Activities

Summary : In this study, some new 3-oxo-5-benzylidene-6-methyl-(4H)-2-substituted pyridazine derivatives have been prepared by the reaction of 5-substituted benzylidene-6-methyl-(4H)-pyridazin-3-one with several substituted benzoyl-methyl bromides. The structures of the compounds have been elucidated by IR, ¹H-NMR and elemental analysis. Antinociceptive (only for compound IIIa) and anti-inflammatory activity studies were evaluated by using in vivo tests. The anti-inflammatory activity was studied by means of the "carrageenan paw edema", whereas the "acetic acid writhing" test was used to assess the antinociceptive activity. Qualitatively, compound IIIa was shown to exert anti-inflammatory effect as potent as phenylbutazone and antinociceptive effect similar to acetylsalicylic acid.

Keywords: 3-Oxo-5-benzylidene-6-methyl-(4H)-2-substituted pyridazines, antinociceptive activity, anti-inflammatory activity, synthesis

Received : 14.4.2003

Revised : 24.6.2003

Accepted : 22.7.2003

Antinosiseptif ve Antiinflatuvar Etkili Bazı 3-Okso-5-benziliden-6-metil-(4H)-2-sübstitüepiridazinler Üzerinde Çalışmalar

Özet: Bu çalışmada, 5-sübstitüebenziliden-6-metil-(4H)-piridazin-3-on türevlerinin değişik sübstitüe benzoilmetil bromürler ile reaksiyonu ile bazı yeni 3-okso-5-benziliden-6-metil-(4H)-2-sübstitüepiridazin türevleri hazırlanmış ve bileşiklerin yapıları IR, ¹H-NMR ve elemental analiz verileriyle kanıtlanmıştır. Antinosiseptif (sadece bileşik IIIa) ve antiinflatuvar aktivite çalışmaları in vivo testlerle yapılmıştır. Antinosiseptif aktivitenin tayininde "asetik asit writhing" testi, antiinflatuvar aktivite tayininde ise "pençe ödem" testi kullanılmıştır. Yapılan aktivite çalışmaları sonucunda, bileşik IIIa'nın fenilbutazona eşdeğer bir antiinflatuvar etki ve asetilsalisilik asite yakın bir antinosiseptif etki gösterdiği bulunmuştur.

Anahtar kelimeler: 3-Okso-5-benziliden-6-metil-(4H)-2-sübstitüepiridazin, antinosiseptif aktivite, antiinflatuvar aktivite, sentez.

1. Introduction

It is well known that the therapeutic use of classical non-steroidal anti-inflammatory drugs (NSAIDs) has a hazardous potential. In view of this fact, research has been directed in recent years at designing compounds devoid of the side effects typical of morphine-like opioid agonists (such as respiratory depression, constipation and physical dependence), as well as of the gastro-intestinal problems. The results of the pharmacological screening of these compounds indicated that some possess good anti-

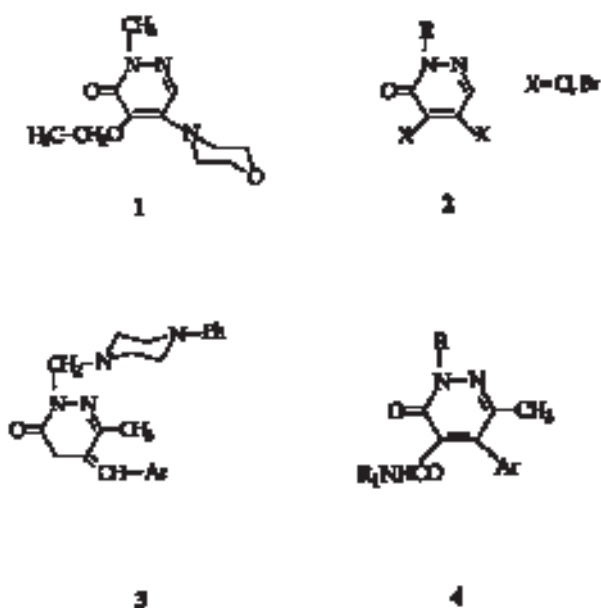
inflammatory activity associated with non-narcotic analgesic properties¹⁻⁴. On the other hand, a considerable number of 3(2H)-pyridazinone derivatives endowed with antinociceptive and antiinflammatory properties have been reported recently⁵⁻¹².

Among these compounds, Emorfazone **1**^{8,9} (which was launched as an analgesic in Japan at the beginning of the last decade), the 4,5-dihaloderivatives **2**¹⁰, the 5-arylidenes **3**⁷ and 4-carbamoylpyridazinones **4**¹¹ have emerged as being of particular interest.

* Hacettepe University, Faculty of Pharmacy, Dept. of Pharmaceutical Chemistry, 06100 Ankara-TURKEY.

** Marmara University, Dept. of Pharmacology and Clinical Pharmacology, The School of Medicine, İstanbul-TURKEY.

^o Corresponding author



Later, Dal Piaz et al. synthesized and evaluated the antinociceptive activities of the compounds having 2-substituted 4,5-functionalized 6-phenyl-3(2H)-pyridazinone structures. They observed that some were more potent than Emorfazone¹³. In addition various compound having a 3(2H)-pyridazinone ring in their structure have been synthesized and their antinociceptive activities have been reported¹⁴⁻¹⁶.

Stimulated by these findings, our attention has been focused on the synthesis of a series of new 3-oxo-5-benzylidene-6-methyl-(4H)-2-substitutedpyridazine derivatives (IIIa-e) which are expected to show antinociceptive and antiinflammatory activities .

2. Materials and methods

2.1. Chemistry

All chemicals used in this study were supplied from E. Merck (Dormstadt, Germany) and Aldrich (Steinheim, Germany). Melting points were determined with a Thomas-Hoover Capillary Melting Point Apparatus (Philadelphia, PA; USA) and are uncorrected. IR spectra (KBr) were recorded on a Perkin-Elmer 1720X (Beaconsfield, UK) FTIR . ¹H-NMR spectra were acquired in CDCl₃ on a Bruker AC 80 MHz FT NMR Instrument (Karlsruhe,

Germany). Tetramethylsilane was used as internal standard and all chemical shift values were recorded as δ (ppm) values. The purity of the compounds was controlled by thin layer chromatography (Merck, silicagel, HF₂₅₄₊₃₆₆, type 60, 0.25 mm, Darmstadt, Germany). The elemental analyses (C, H, N, and S) were performed on a Leco CHNS 932 (Leco Cooperation, St. Joseph, MI, USA) analyzer by the Scientific and Technical Research Council of Turkey Instrumental Analysis Laboratories (Ankara, Turkey) and were within ± 0.4 % of the theoretical values.

2.1.1. General preparation of 4-nonsubstituted/-substituted benzoylmethyl bromides

These compounds were synthesized by treating appropriate acetophenones with bromine in glacial acetic acid according to the method reported earlier¹⁷.

2.1.2. Preparation of 4-oxo-3-benzylidenepentanoic acid (I)

An ice-cooled mixture of benzaldehyde (0.2 mol) and levulinic acid (0.3 mol) was saturated with dry hydrogen chloride. Then, the mixture was stirred for 48 h at room temperature. The precipitate which formed was filtered off . The crude acid was crystallized from ethyl acetate/n-hexane mixture.

2.1.3. Preparation of 5-benzylidene-6-methyl-(4H)-pyridazin-3-one (II)

A mixture of acid (I) (0.02 mol) and hydrazine hydrate (0.02 mol) in ethanol (50 ml) was refluxed for 2h. Then the mixture was cooled and the crude product which separated was filtered off and crystallized from ethanol.

2.1.4. General preparation of 3-Oxo-5-benzylidene-6-methyl-(4H)-2-substituted pyridazines (IIIa-e)

5-Benzylidene-6-methyl-(4H)-pyridazin-3-one (0.01mol) was added to an ethanolic solution (25 ml) of sodium (0.23 g, 0.01 g atom). The mixture was refluxed for 30 min. Then, appropriate benzoyl-

methyl bromides (0.01 mol) were added by drops to a cooled solution, which was refluxed for 48 h and evaporated in vacuo. The crude product was crystallized from ethyl acetate/n-hexan.

3-Oxo-5-benzylidene-6-methyl-(4H)-2-(benzoylmethyl)pyridazine (IIIa)

This was obtained from 0.01mol (2 g) 5-benzylidene-6-methyl-(4H)-pyridazin-3-one and 0.01 mol (1.99 g) benzoylmethyl bromide. Yield 65%. M.p.:170-1 °C. IR: 1660 (C=O), ¹H-NMR: 2.2 (3H, s, CH₃-pyr), 3.8 (2H, s, -CO-CH₂-), 5.5 (2H, s, -N-CO-CH₂-), 6.6 (1H, s, -CH=C-), 7.0-8.2 (10H, m, aromatic)

3-Oxo-5-benzylidene-6-methyl-(4H)-2-(4-methylbenzoylmethyl) pyridazine (IIIb)

This was obtained from 0.01mol (2 g) 5-benzylidene-6-methyl-(4H)-pyridazin-3-one and 0.01 mol (2.13 g) 4-methylbenzoylmethyl bromide. Yield 68%. M.p.:118-9 °C. IR:1665(C=O), ¹H-NMR: 2.2 (3H, s, CH₃-), 2.4 (3H, s, CH₃-), 3.8 (2H, s, -CO-CH₂-), 5.5 (2H, s, -N-CO-CH₂-), 6.5 (1H, s, -CH=C-), 7.0-8.2 (9H, m, aromatic)

3-Oxo-5-benzylidene-6-methyl-(4H)-2-(4-methoxybenzoylmethyl) pyridazine (IIIc)

It was obtained from 0.01mol (2 g) 5-benzylidene-6-methyl-(4H)-pyridazin-3-one and 0.01 mol (2.29 g) 4-methoxybenzoylmethyl bromide. Yield 70%. M.p.:131-2 °C. IR:1670 (C=O), ¹H-NMR: 2.2 (3H, s, CH₃-pyr), 3.65 (2H, s, -CO-CH₂-), 3.95 (3H, s, CH₃O-ph), 5.4 (2H, s, -N-CO-CH₂-), 6.5 (1H, s, -CH=C-), 6.9-8.1 (9H, m, aromatic)

3-Oxo-5-benzylidene-6-methyl-(4H)-2-(4-bromobenzoylmethyl) pyridazine (III d)

It was obtained from 0.01mol (2 g) 5-benzylidene-6-methyl-(4H)-pyridazin-3-one and 0.01 mol (2.78 g) 4-bromobenzoylmethyl bromide. Yield 62%. M.p.: 137-8 °C. IR:1660 (C=O), ¹H-NMR: 2.2 (3H, s, CH₃-pyr), 3.65 (2H, s, -CO-CH₂-), 5.4 (2H, s, -N-CO-CH₂-), 6.45 (1H, s, -CH=C-), 6.9-7.9 (9H, m, aromatic)

3-Oxo-5-benzylidene-6-methyl-(4H)-2-(4-chlorobenzoylmethyl) pyridazine (IIIe)

This was obtained from 0.01mol (2 g) 5-benzylidene-6-methyl-(4H)-pyridazin-3-one and 0.01 mol (2.33 g) 4-chlorobenzoylmethyl bromide. Yield 64%. M.p.: 113-4 °C. IR:1665 (C=O), ¹H-NMR: 2.1 (3H, s, CH₃-pyr), 3.7 (2H, s, -CO-CH₂-), 5.4 (2H, s, -N-CO-CH₂-), 6.5 (1H, s, -CH=C-), 6.9-8.0 (9H, m, aromatic)

2.2. Biological Evaluation

2.2.1. Animals

Swiss Albino mice of 20-25 g (Marmara University, Experimental Research and Animal Laboratory) were used for examining the anti-inflammatory and antinociceptive activities of the compounds. All experiments were carried out by humane methods and with the approval of Hacettepe University Ethical Committee for Experimental Animals. The animals were kept in a temperature-controlled, 12-h light and dark medium and fed with standard rodent pellet and water. All of the mice were sacrificed by cervical dislocation after the completion of experiments.

2.2.2. Drug Solutions

All drugs were suspended in 0.5 % carboxymethyl cellulose Na (CMC Na, Aldrich, Steinheim, Germany) solution and mixed homogeneously in an ultrasonic bath.

2.2.3. Anti-inflammatory activity

The activity was performed following the technique of Winter et al.¹⁸. Either the test compounds and the vehicle carboxymethyl cellulose or the reference drug phenylbutazone (Aldrich, Steinheim, Germany), were administered orally at a dose of 100 mg/kg. The drug solutions were administered within a volume of 0.1 ml/10 g of mice. Two % carageenan solution (Sigma, St. Louis, MO, USA) freshly prepared in 0.5 % CMC Na was injected into

the plantar aponeurosis of the right paw of mice 1 h following administration of pre-treatment drugs. The thickness of the paw was measured by a micrometer with a sensitivity of 0.05 mm prior to the injection of carrageenan and 2 h later. Percent change in paw thickness was calculated according to the following formula:

Percent change in paw thickness = $(t' - t / t) \times 100$
 t = the paw thickness prior to carrageenan injection
 t' = the paw thickness measured 2 h following carrageenan injection

The reference drug was also administered at 25 mg/kg and 50 mg/kg doses for calculation of ED₅₀ values, likewise most active compound.

2.2.4. Antinociceptive activity

"Acetic acid writhing" test¹⁹ was used for testing antinociceptive activity. Carboxymethyl cellulose sodium (CMC Na, Aldrich, Steinheim, Germany), acetyl salicylic acid (Bayer, Istanbul, Turkey) and the test compound IIIa were given orally at 100 mg/kg dose as a suspension in 0.2 ml of 0.5 % CMC Na to groups of 5 mice of both sexes, pregnant females excluded. Control animals received the same volume of vehicles. 1 h after the drug administration, every mouse was treated with an aqueous acetic acid solution (3 % w/v, Merck, Darmstadt, Germany) injected intraperitoneally at a volume of 10 ml/kg dose. 5 min following the administration of acetic acid solution, the number of stretching produced in the lower extremities was recorded for a duration of 10 min. The mean number of writhes for each experimental group was calculated. The data were then converted into % antinociceptive activity by using the formula below:

Percent antinociceptive activity = $[(v - v') / v] \times 100$
 v = number of writhes in vehicle treated mice
 v' = number of writhes in drug-treated mice.

Lower doses (25 mg/kg and 50 mg/kg) of the reference and the most active test compound were also examined for calculation of ED₅₀ values.

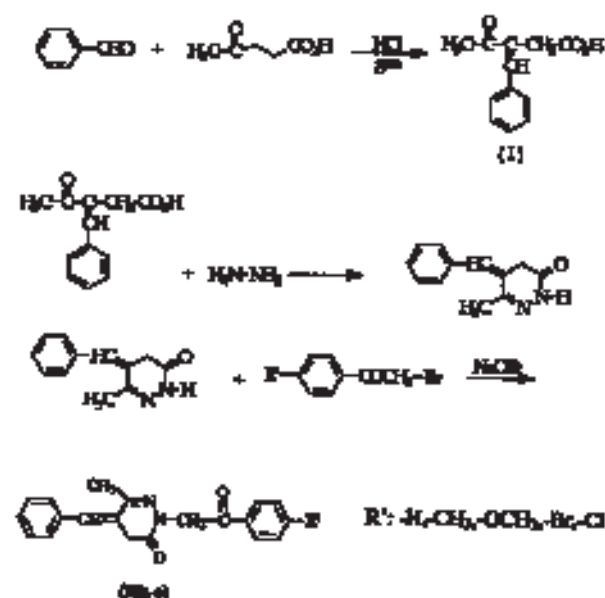
2.2.5. Statistical analysis

Results were expressed as means \pm s.e.m. Statistical significance was analysed using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where $p < 0.05$ was accepted to be a significant difference. ED₅₀ values and 95 % confidence limits (95 % CL) were calculated from the dose-percent inhibition relations by computer log-linear regression analysis.

3. Results

3.1. Synthesis

In the first step of our studies, 4-oxo-3-benzylidene-pentanoic acid(I) was prepared by reacting benzaldehyde with levulinic acid in the presence of dry hydrogen chloride under the reaction conditions described earlier by Rubat et al.²⁰. Then this compound was converted into 5-benzylidene-6-methyl-(4H)-pyridazin-3-one (II), by treating with hydrazine hydrate²⁰. In the last step of our reactions, several substituted benzoylmethyl bromides were reacted with 5-substituted benzylidene-6-methyl-(4H)-pyridazin-3-one to obtain target compounds, 3-oxo-5-benzylidene-6-methyl-(4H)-2-(4-nonsubstituted/-substituted benzoylmethyl)pyridazines (IIIa-e) (Scheme 1). The chemical structures of the synthesized compounds were confirmed using the spectroscopic techniques such as IR, ¹H-NMR, and elementary analysis results.



Scheme 1. Synthetic pathway of the compounds

3.2. Biological Testing

All compounds were subjected to in vivo tests in order to evaluate their pharmacological activity. The anti-inflammatory activity was studied by means of the "carrageenan paw edema", whereas the "acetic acid writhing" test was used to assess the antinociceptive activity. The most active compounds were investigated in details for their ED₅₀ values.

The anti-inflammatory activities of test compounds at 100 mg/kg dose were compared with the same dose of the reference drug phenylbutazone (PBZ). The percent increase in paw thickness measured in carboxymethyl cellulose and phenylbutazone pre-treated mice were found to be 50 ± 8 and 122 ± 7, respectively. Pre-treatment with all of the test compounds increased the paw thickness, but the percent increase in IIIa pre-treated mice (56 ± 11) was smaller than IIIb, IIIc, IIIId and IIIe. Statistical comparison with one-way analysis of variance followed by Tukey's multiple comparison test revealed that percent change in paw thickness in mice pre-treated with phenylbutazone and IIIa were significantly different from the percent change in mice that received carboxymethyl cellulose ($p < 0.05$; Figure 1). For phenylbutazone and IIIa, the 25 mg/kg and 50 mg/kg doses were also tested (Figure 2). The ED₅₀ values of phenylbutazone and IIIa were calculated as 113 mg/kg (95% confidence interval: 65-127 mg/kg) and 112 mg/kg (95% confidence interval: 66-124 mg/kg), respectively. These results show that compound IIIa is as much potent as phenylbutazone.

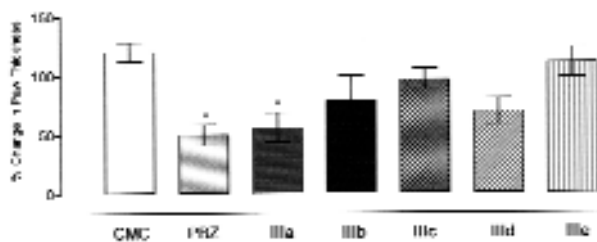


Figure 1. The percent change in paw thickness of mice measured before and 2 h following 2 % carrageenan solution into the plantar aponeurosis of right paw. The pre-treatments were given orally 1 h before the measurements. $p < 0.05$, compared to the group receiving carboxymethyl cellulose sodium.

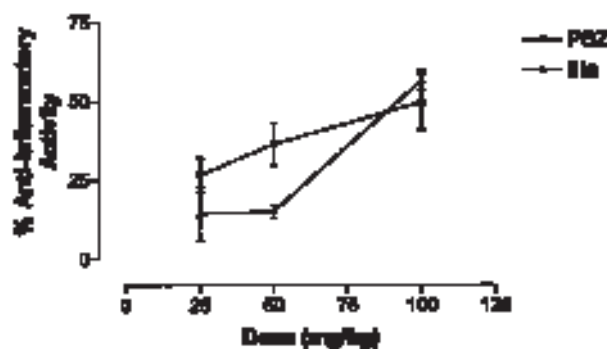


Figure 2. The percent anti-inflammatory activity of phenylbutazone (PBZ) and test compound IIIa orally administered at 25 mg/kg, 50 mg/kg and 100 mg/kg doses.

The compound IIIa that was found to possess anti-inflammatory activity in the screening test was studied for antinociceptive activity using "acetic acid writhing" test (modified Koster's test). Acetylsalicylic acid (ASA) was used as reference drug. CMC Na was also administered as a control group. All the compounds were tested at a dose level of 100 mg/kg. The number of stretching behaviour occurring in response to intraperitoneal administration of acetic acid was found to be larger in the group treated with carboxymethyl cellulose than the groups treated either with the acetylsalicylic acid or IIIa (Figure 3). Statistical analysis revealed that IIIa and CMC Na groups produced a significant difference ($p < 0.05$). In addition, comparison of acetyl salicylic acid and IIIa was not found to be significantly different. In figure 4, the percent antinociceptive activity in response to 3 different doses of ASA and IIIa were demonstrated. The ED₅₀ values of acetylsalicylic acid and IIIa were calculated as 72 mg/kg (95% confidence interval: 47.5-97 mg/kg) and 82 mg/kg (95% confidence interval: 58-132 mg/kg), respectively. These results show that compound IIIa is as potent as acetylsalicylic acid in terms of antinociception.

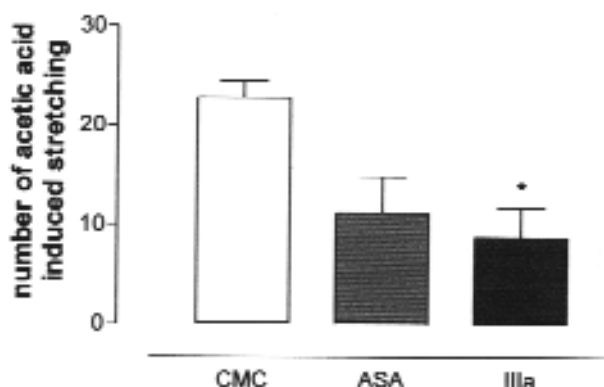


Figure 3. The number of acetic acid induced stretching in mice that occurred within 10 min. The observation was started 5 min following administration of intraperitoneal acetic acid. The pre-treatments were given orally 1 h before the measurements. $p < 0.05$, compared to the group receiving carboxymethyl cellulose sodium.

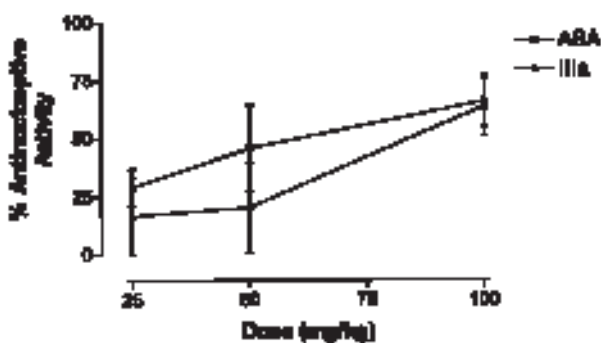


Figure 4. The percent antinociceptive activity of acetyl salicylic acid (ASA) and test compound IIIa administered at 25 mg/kg, 50 mg/kg and 100 mg/kg doses via oral route.

4. Discussion

In the IR spectra of the compounds IIIa-e, it was seen that the carbonyl absorption bands belonging to pyridazine ring and ketone group overlapped at about 1660 cm^{-1} . Furthermore, N-H stretching band belonging to the 5-benzylidene-6-methyl-(4H)-pyridazin-3-one ring disappeared because of N-substitution. In the $^1\text{H-NMR}$ spectra of compounds IIIa-e the signal appearing at around 3.7 ppm as singlet proved the presence of a benzoylmethyl moiety.

It has been proposed that anti-inflammatory activity is a consequence of inhibition of cyclooxygenase

that catalyses conversion of arachidonic acid into prostaglandins^{21,22}. In this study, the pharmacological experiments performed using carrageenan test showed that IIIa possessed an anti-inflammatory activity. In the present study, the antinociceptive effect of compound IIIa was also found to be almost equipotent with acetyl salicylic acid. The comparison of ED_{50} values of compound IIIa and phenylbutazone in terms of anti-inflammatory effects revealed that compound IIIa is as potent as the reference drug. Phenylbutazone, a member of the pyrazolone group non-steroidal anti-inflammatory drug is very effective in exerting anti-inflammatory effects but its hematological adverse reactions limited its use²³. The mechanism of anti-inflammatory activity of compound IIIa should be examined further, one of which is the inhibition of cyclooxygenase. Although inhibition of prostaglandin synthesis is attributed to be the main mechanism of anti-inflammatory activity, radioligand binding experiments showed that some non-steroidal anti-inflammatory agents possess intrinsic glucocorticoid receptor agonist activity²⁴. Inhibition of superoxide formation by non-steroidal anti-inflammatory drugs is also another pathway to exert the anti-inflammatory effect²⁵. Collectively, several investigators forwarded central nervous system mechanisms as one of the modes of action of some non-steroidal anti-inflammatory drugs. It was demonstrated that activation of inhibitory pathways originating from periaqueductal gray matter to the spinal cord mediates central analgesic effects of metamizol²⁶. Since opioids and synthetic analogues affect the receptors present either in the spinal cord or brain, the antinociceptive activity of these compounds and the agents having a similar mechanism of action should be screened by using different experimental setting using various antagonists like naloxone and different reference drugs like fentanyl or meperidin (pethidine).

The anti-inflammatory activity of compound IIIb was found to be less than IIIa, so the antinociceptive activity of IIIb was not tested using the acetic acid induced writhing test. IIIc, IIIe and IIIe did not exert an anti-inflammatory effect, so their antinoci-

ceptive activities were not evaluated using acetic acid induced stretching behavior. In conclusion, compound IIIa has anti-inflammatory effect as potent as phenylbutazone and antinociceptive activity quantitatively similar to acetylsalicylic acid.

Acknowledgement

The authors gratefully acknowledge the financial support of the Research Fund of Hacettepe University through the grant AFP 00 02 301 006.

References

1. Russo F, Guccione S, Santagati NA, Santagati A, Caruso A, Leone MG, Felice A, Attaguile G, Roxas MA. New hetero cyclic ring systems - V synthesis and pharmacological activity of 6H-1,3,4-thiadiazolo[3'2':1,2]-5-oxopyrimido[5,4-b] indole derivatives and of 1-phenyl-6H-1,2,4-triazolo[1'5':1,2]-5-oxopyrimido[5,4-b]indole. *IL Farmaco*, 43, 409-420, 1988.
2. Russo F, Romeo G, Guccione S, Bousquet E, Caruso A, Leone MG, Attaguile G, Roxas MA. Synthesis and pharmacological activity of 6H-thiazolo[3',2':1,2]5-oxopyrimido[5,4-b]indole derivatives a new hetero cyclic ring system, *Pharmazie*, 45, 242-244, 1990.
3. Russo F, Guccione S, Romeo G, Monsu'scolaro L, Pucci S, Caruso A, Cutuli V, Roxas MA, Pyrazolo thiazolopyrimidine derivatives as a novel class of anti-inflammatory or antinociceptive agents: synthesis structural characterization and pharmacological evaluation, *Eur. J. Med. Chem.*, 27, 73-80, 1992.
4. Russo F, Guccione S, Romeo G, Barretta GU, Pucci S, Caruso A, Roxas MA, Cutuli V, Research on heterocyclic compounds XXXI. Synthesis and anti-inflammatory activity of 2-arylimidazo[1,2a]pyrazine-3-carboxylic acids, *Eur. J. Med. Chem.*, 28, 363-376, 1993.
5. Rubat C, Coudert P, Couquelet J, Bastide P, Bastide J. Synthesis and analgesic effect of N-substituted 5-arylidene-6-methyl-3-(3H)-pyridazinones, *Chem Pharm Bull*, 36(4), 1558-1561, 1988.
6. Nannini G, Biasoli G, Perrone E, Forgiione A, Buttnoni A, Ferrari M. Synthesis and pharmacological activity of some 5,6-diphenyl-pyridazines, *Eur J Med Chem-Chim Ther.*, 14(1), 53-60, 1979.
7. Rubat C, Coudert P, Albuisson E, Bastide J, Couquelet J, Tronche P. Synthesis of mannich bases of arylidenepyridazinones as analgesic agents, *Journal of Pharmaceutical Sciences*, 81, 1084-1087, 1992.
8. Takaya M, Sato M, Terashima K, Tanizawa H. A new nonsteroidal analgesic-antiinflammatory agent. Synthesis and activity of 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone and related compounds, *Journal of medicinal chemistry*, 22(1), 53-58, 1979.
9. Sato M, Ishizaku Y, Yamaguchi A. Pharmacological investigations of 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (M 73101), a new analgesic and antiinflammatory drug, *Arzneim Forsch*, 31, 1738-1745, 1981.
10. Santagati NA, Duro F, Caruso A, Trombadore S, Amico-Roxas M. Synthesis and pharmacological study of a series of 3(2H)-pyridazinones as analgesic and antiinflammatory agents, *Il Farmaco*, 40, 921-929, 1985.
11. Pinna GA, Sardu F, Curzu MM. Synthesis and pharmacological activity of 4-carbamoyl-5-aryl-6-methyl-4,5-dihydropyridazin-3(2H)-ones, *Il Farmaco*, 47, 449-463, 1992.
12. Flouzat C, Bresson Y, Mattio A. Novel non opioid non-antiinflammatory analgesics : 3-(aminoalkyl) and 3-[(4-aryl-1-piperazinyl)alkyl]oxazolo [4,5-b] pyridin-2(3H)ones, *J. Med. Chem.*, 36(4), 497-503, 1993.
13. Piaz VD, Giovannoni MP, Ciciani G, Barlocco D, Giardina G, Petrone G, Clarke GD. 4,5-Functionalized 6-phenyl-3(2H)-pyridazinones:synthesis and evaluation of antinociceptive activity, *Eur. J. Med. Chem.*, 31, 65-70, 1996.
14. Doğruer DS, Şahin MF, Ünlü S, Ito S. Studies on some 3(2H)-pyridazinone derivatives with antinociceptive activity, *Arch.Pharm.Pharm.Med.Chem*, 333, 79-86, 2000.
15. Giovannoni MP, Vergelli C, Ghelardini C, Galeotti N, Bartolini A, Piaz VD. [(3-Chlorophenyl)piperazinylpropyl]pyridazinones and analogues as potent antinociceptive agents, *J Med Chem*, 46, 1055-1059, 2003.
16. Gökçe M, Doğruer D, Şahin MF. Synthesis and antinociceptive activity of 6-substituted-3-pyridazinone derivatives, *Il Farmaco*, 56, 233-237, 2001.
17. İmmediata T, Day AR. β-Naphthyl derivatives of ethanolamine and N-substituted ethanolamines, *J. Org. Chem.*, 5, 512-527, 1940.
18. Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paw of rat as an assay for anti-inflammatory drugs, *Proc. Soc. Exp. Biol. Med.*, 111, 544-547, 1962.
19. Davies JE, Kellett DN, Pennington JC. The anti-inflammatory and analgesic effects of norvedan a novel, *Arch. Int. Pharmacodyn. Ther.*, 221, 274-282, 1976.
20. Rubat C, Coudert P, Refouvelet B, Tronche P, Bastide P, Bastide J. Anticonvulsant activity of 3-oxo-5-substituted benzylidene-6-methyl-(4H)-2-pyridazinylacetamides and 2-pyridazinylacetylhydrazides, *Chem Pharm Bull*, 38(11), 3009-3013, 1990.
21. Famaey JP. Recent developments about non-steroidal anti-inflammatory drugs and their mode of action, *Gen. Pharmacol.*, 9, 155-162, 1978.
22. Day RO. Mode of action of non-steroidal anti-inflammatory drugs. *Med J Austral*, 148, 195-200, 1988.
23. Friedman GD. Phenylbutazone, musculoskeletal disease and leukemia, *J. Chron. Dis.*, 35, 233-238, 1982.
24. Feldman D. Binding of some non-steroidal anti-inflammatory drugs to glucocorticoid receptors in vitro, *Biochemical Pharmacology*, 27, 1187-1191, 1978.
25. Oyanagui Y. Inhibition of superoxide anion production in macrophages by anti-inflammatory drugs, *Biochemical Pharmacology*, 25, 1473-1480, 1976.
26. Carlsson KH, Helmreich J, Jurna I. Activation of inhibition from the periaqueductal gray matter mediates central analgesic effects of metamizol (dipyrone), *Pain*, 27, 373-390, 1986.