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RESEARCH ARTICLE

Synthesis and Evaluation of the Anticonvulsant Activities of Some 5-(4-substitutedbenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-ones

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Synthesis and evaluation of the anticonvulsant activities of some 5-(4-substitutedbenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-ones

Summary

In this study, starting from 3-benzylidene-4-oxopentanoic acid derivatives (1a-e), having 5-benzylidene-6-methyl-4,5-dihydropyridazine-3(2H)-one (2a-e) structure and their ester (3a-e), hydrazide (4a-e) and acetic acid (5a-e) derivatives were synthesized. The physical properties and UV absorptions of the five starting compounds (1a-e) and twenty target compounds (2a-5e) were determined. Their chemical structures were achieved by IR and ^{1}H-NMR spectral data. Additionally, elemental analysis data of the new compounds (1b, 2b, 3b, 4b, 5b, 3c, 4c, 5c, 2e, 3e, 4e and 5e) were done to identify the structures. Anticonvulsant activities of the target compounds (2a-5e) were screened by pentylenetetrazole seizure model. It was observed that compound 2a showed an anticonvulsant effect similar to ethosuximide concerning the seizure grade

Key Words : *4,5-dihydropyridazine-3(2H)-one, 6-oxo-5,6-dihydropyridazinylacetate, 6-oxo-5,6-dihydropyridazinylacetohydrazide, 6-oxo-5,6-dihydropyridazinylacetic acid, anticonvulsant activity*

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Bazı 5-(4-sübstitütebenziliden)-6-metil-4,5-dihidropiridazin-3(2H)-on Türevlerinin Sentezleri ve Antikonvülsan Aktiviteleri

Özet

Bu çalışmada, 3-benziliden-4-oksopentanoik asit türevlerinden (1a-e) hareketle 5-benzilidene-6-metil-4,5-dihidropiridazin-3(2H)-on (2a-e) yapısındaki beş bileşikle, bunların ester (3a-e), hidrazit (4a-e) ve asetik asit (5a-e) türevleri olan on beş bileşik sentezlenmiştir. Sentezleri yapılan beş hareket maddesiyle (1a-e) yirmi sonuç bileşiğin (2a-5e) fiziksel özellikleri ve UV absorbsiyon özellikleri saptanmış, yapıları IR ve ^{1}H-NMR spektrumları yardımıyla kanıtlanmıştır. Literatürde kayıtlı olmayan 12 yeni bileşiğin (1b, 2b, 3b, 4b, 5b, 3c, 4c, 5c, 2e, 3e, 4e ve 5e) yapıları, yukarıda bildirilen analitik yöntemlerin yanısıra elementel analiz verileri ile desteklenmiştir. Yapıları kanıtlanan sonuç bileşiklerin (2a-5e) antikonvülsan aktiviteleri "pentilentetrazol tarama testi" kullanılarak incelenmiş ve 2a bileşiğinin etosüksimide benzer antikonvülsan aktiviteye sahip olduğu gözlenmiştir.

Anahtar Kelimeler : *4,5-dihidropiridazin-3(2H)-on, 6-okso-5,6-dihidropiridazinilasetat, 6-okso-5,6-dihidropiridazinilasetohidrazit, 6-okso-5,6-dihidropiridazinilasetik asit, antikonvülsan aktivite*

INTRODUCTION

Many papers have reported that the compounds having pyridazinone ring in their structures possess antinociceptive[1-3], antiinflammatory[4-6], anticonvulsant[7-11] aldose reductase inhibitory[12] and antihypertensive[13] activities. Rubat et al.[10] described the anticonvulsant activities of 5-substitutedbenzylidene-6-methyl-4,5-dihydropyridazine-3(2H)-one derivatives. According to them, the most common structural elements of clinically active drugs against epilepsy appeared to be a nitrogen heteroatomic system be-

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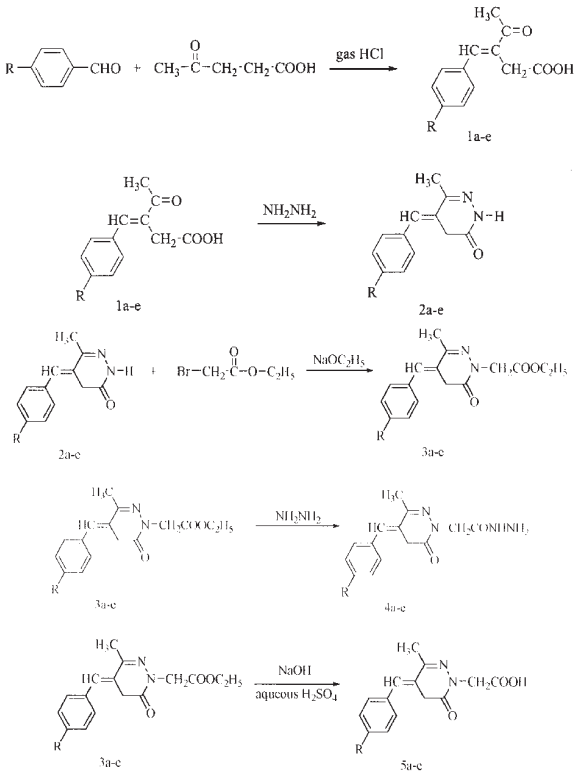
aring one or two phenyl rings and at least one carbonyl group. In order to agree with these structural characteristics they synthesized 3-ureido, 3-semicarbazido and 3-hydrazidopyridazine derivatives. These observations prompted us to synthesize a series of 5-(4-substitutedbenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one and their substituted derivatives and to further explore the antiepileptic potential of these compounds in a different seizure model in which motor seizures are induced by subcutaneous injection of pentylenetetrazole (PTZ; 'threshold seizure test'). This is a widely used antiepileptic screening test accepted as a model for generalized seizures of the myoclonic type.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic route of the target compounds is outlined in Scheme 1. The starting compounds, 3-benzylidene-4-oxopentanoic acid derivatives (1a-e), were prepared by reacting appropriate benzaldehydes with levulinic acid according to the method reported earlier[10,14]. 4-Oxo-3-arylidenepentanoic acids (1a-e) and hydrazine hydrate were refluxed to obtain 5-benzylidene-6-methyl-4,5-dihydropyridazine-3(2H)-one derivatives (2a-e) according to Taoufik and coworkers' method[19]. The synthesis of ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetates, (3a-e) was performed by reacting 5-benzylidene-6-methyl-4,5-dihydropyridazine-3(2H)-one (2a-e) with ethyl bromoacetate in the presence of sodium ethoxyde according to the method described earlier by Rubat et al.[10]. By the reaction of ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetates, (3a-e) with hydrazine hydrate, [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl] acetohydrazide derivatives (4a-e)[10], and by the hydrolysis of these compounds (3a-e) with sodium hydroxide, [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid derivatives (5a-e)[12] were obtained.



R: a -H, b -Br, c -Cl, d -CH_3, e -OCH_3

Scheme 1. Synthesis of the compounds.

Although the synthesis of some compounds (2a, 2c, 2d, 3a, 3d, 4a, 4d, 5a and 5d) in the series were given before, these compounds were synthesized in our study according to the methods in the literatures, in order to investigate the effects of the different substituents on the anticonvulsant activity. The melting points of these compounds are in accordance with the literature.

The structures of the synthesized compounds were in accordance with their elementary analysis and spectral data. In the IR spectra of the starting compounds, (1a-e), the band seen between 3300-2800 cm^{-1} (O-H stretching) and at about 1790 cm^{-1} (C=O stretching) confirmed the presence of carboxylic acid group. In the IR spectra of 2a-e, C=O stretching band shifted to around 1660 cm^{-1} because of the ring cyclization. On the other hand, in the IR spectra of these compounds, keto-enol tautomerism of the pyridazinone ring caused a broad band to appear between 3200-2850 cm^{-1} (N-H and O-H stretching). In the IR spectra of 3a-e, this broad band disappeared. This si-

tuation can be explained by the nucleophilic substitution of the hydrogen atom, attached on the 2nd position of the pyridazinone ring by ethyl bromoacetate. Furthermore, in the IR spectra of these compounds, a new band, at around 1745 cm^{-1} belonging to ester group (C=O stretching) was seen. In the IR spectra of 4a-e, due to hydrazide group, two sharp N-H stretching bands were observed at around 3308 and 3205 cm^{-1} respectively. In the IR spectra of 5a-e, conversion of the ester group to carboxylic acid caused a broad band to appear between 3100-2750 cm^{-1} (O-H stretching) and shifted C=O stretching band to around 1730 cm^{-1}.

In the ^{1}H-NMR spectra of 1a-e, the signals of the methyl, methylene and methine protons were observed at about 2.50, 3.40 and 7.90 ppm, respectively. In the ^{1}H-NMR spectra of 2a-e, the signal of the proton on nitrogen atom at the 2nd position of the pyridazinone ring was observed at around 12.65 ppm as a singlet. In the ^{1}H-NMR spectra of 3a-e, this signal disappeared because of N-substitution. Furthermore, the triplet appeared at around 1.20 ($-CH_2CO-OCH_2CH_3$), the quartet at around 4.14 ($-CH_2CO-OCH_2CH_3$) and the singlet at around 4.76 ppm ($-CH_2COOCH_2CH_3$) proved the ester group substitution to the pyridazinone ring. In the ^{1}H-NMR spectra of 4a-e, the signals observed at around 4.43 ($NHNH_2$) and 9.24 ppm ($NHNH_2$) proved the conversion of ester group into acetohydrazide group. In the ^{1}H-NMR spectra of 5a-e, the signals belonging to ethyl protons were not observed. Instead of these signals, at around 12.65 ppm, the signal of carboxylic acid proton appeared. These observations were in accordance with the literature data[5,12,14,15].

2.2. Pharmacology

Substances and the reference drugs at the 100 mg/kg dose were screened using PTZ (pentylenetetrazole) test (75 mg/kg) initially. The time that elapsed until the most severe seizure grade, expressed as latency, and the most severe seizure grade observed were noted as shown in Figure 1 and Figure 2, respectively. DPH (diphenylhydantoin), CBZ (carbamazepine), and ETX (ethosuximide) were used as the reference drugs.

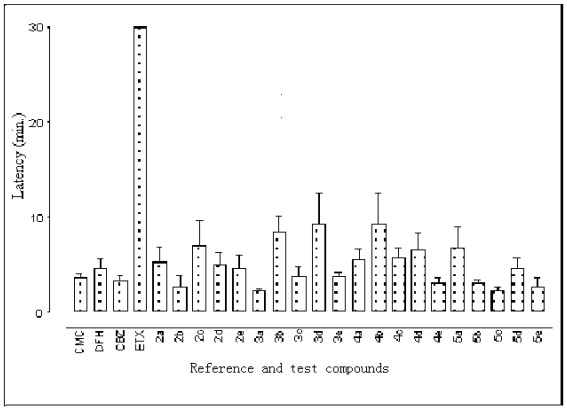


Figure 1. The latency for the most prominent grades of seizures induced by PTZ model in mice where vehicle (CMC, carboxymethylcellulose), reference drugs (DPH, CBZ and ETX), and the test compounds were administered per orally. 30 min was accepted as the cut-off time. In ETX-treated mice no seizure activity was observed within 30 min. The values are expressed as mean ± SEM (n= 4-8).

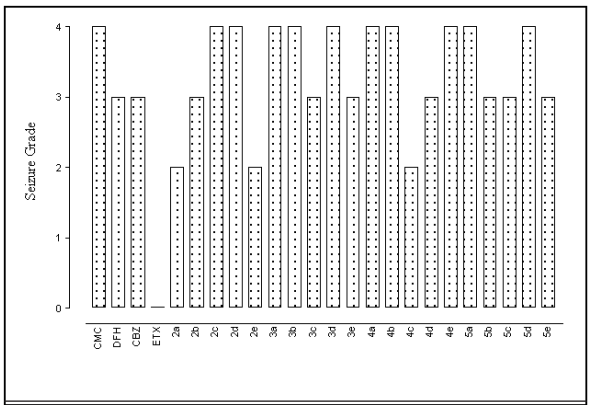


Figure 2. The median values of maximum grade scores of mice pretreated per orally with CMC, reference drugs (DPH, CBZ and ETX) and the test compounds (n=4-8) in response to intraperitoneal pentylenetetrazole injection challenge (75 mg/kg) in PTZ model of seizures.

The substances that were observed to be as potent as the reference CBZ were further tested at 50 mg/kg and 150 mg/kg doses, since no compound was detected to exert an effect to the same extent as ETX. Figure 3 shows the latencies of compounds at three

different doses. Figure 4 shows the maximum grades of mice treated with different doses of compounds. Median values of maximum seizure grades after administration of maximum doses (150 mg/kg) of compounds and reference drug, ethosuximide, are shown in Figure 5.

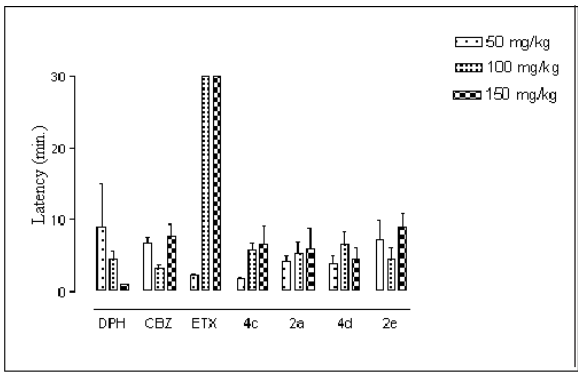


Figure 3. The latency required for the onset of maximum seizure grades in mice pretreated with the reference drugs and the test compounds following intraperitoneal pentylenetetrazole injection. The data are expressed as mean ± SEM (n=4-8).

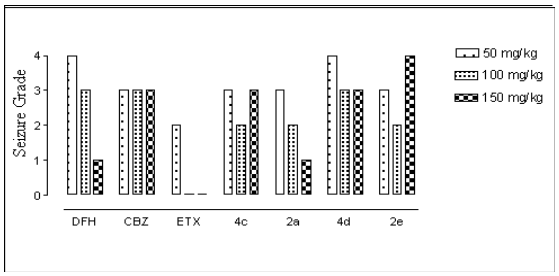


Figure 4. The median values of maximum seizure grades noted in mice pretreated with the reference drugs and the test compounds following intraperitoneal pentylenetetrazole injection (n=4-8).

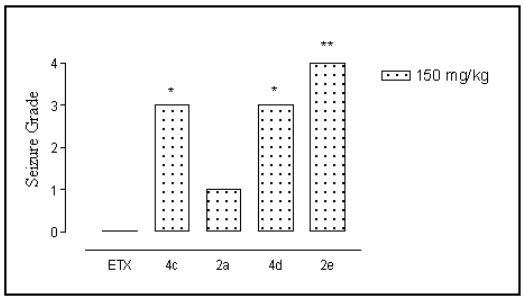


Figure 5. The median values of maximum seizure grades after administration of 150 mg/kg doses of compounds and reference drug, ethosuximide, per orally in pentylenetetrazole test (n=4-8).*, $p<0.05$; **, $p<0.01$ (compared with ETX group).

3. CONCLUSION

Epilepsy, one of the most common neurological disorders, affects about 4% of individiuals over their lifetime[16].

In the absence of a specific etiological understanding, approaches to drug therapy of epilepsy must necessarily be directed at the control of symptoms, i.e. the suppression of seizures[17].

The novel AEDs have brought significant advancement to the treatment of epilepsy; many of the novel AEDs, such as gabapentin, lamotrigine, and oxcarbamazepine, are better tolerated and similarly effective compared to older AEDs such as CBZ or DPH. The new AEDs such as levetiracetam or tiagabine have widened the choice of good AEDs for the treatment of newly diagnosed epilepsies and have been very beneficial for patients in whom older first-line AEDs were not tolerated well or did not control seizures.

DPH, CBZ and ETX were used as the reference drugs in this study, where the results revealed that DPH and CBZ are not effective against PTZ-induced seizures and that ETX is the most appropriate reference drug in the PTZ screening test. This finding is in accordance with the literature demonstrating that ETX is suitable in generalized seizures. It also validates the PTZ model as a suitable model for generalized seizures and our experimental settings. Apart from the bromides and phenobarbital, the anticonvulsant effect of all first and second generation AEDs was first determined in animal models, such as the maximal electroshock seizure (MES) or the PTZ seizure tests in mice or rats, demonstrating that clinical activity can be predicted by such simple laboratory models[18]. Therefore, seizure models in laboratory animals are still the most important prerequisite in the preclinical search for new AEDs.

If the median seizure grades of the test compounds and ETX administered at 150 mg/kg dose were compared, significant statistical differences between 4c, 4d and 2e were found; however, no statistically

significant difference was found with the test compound 2a. This finding may denote that substance 2a has an anticonvulsant effect similar to that of ETX concerning the seizure grade. However, if the latency is taken into consideration, 2a was not as good as ETX. Nevertheless, this conflicting property, the inhibition of progression of seizure grade to a higher level, rather than its effect on the initiation of the seizures, may give substance 2a a different characteristic, i.e. for use in status epilepticus, the most dramatic clinical situation. Pentylenetetrazole can also be used in the status epilepticus studies. In our experimental design we could only administer the agents orally since the compounds were not soluble for parenteral administration. If such modification can be made, the effect of substance 2a should be further tested in a different setting using the suitable dose of PTZ for status epilepticus.

In addition, some of the new AEDs such as gabapentin, levetiracetam or tiagabine have significantly better pharmacokinetics and are not involved in drug-drug interactions. Futhermore, some of the new AEDs do not seem to share the teratogenic potential of older AEDs. We did not observe any toxic reactions during the screening experiments, but as expected it is not a guarantee for its safety; many toxicological examinations of the compounds should also be carried out together with the pharmacokinetic studies. The compound 2a and the others should also be further tested using other screening methods like maximal electroshock model. The compounds exhibiting a promising effect can also be extensively tested in very specific models, such as kindling model of epilepsy and genetic absence epilepsy model.

We can assume that the pyridazinone derivatives having ester, hydrazide and acid groups on the nitrogen atom were not more active than the nonsubstituted derivatives. On the other hand, some acetohydrazide derivatives such as 4c and 4d were found more active than the corresponding ester and acid derivatives.

4. EXPERIMENTAL

4.1. 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 Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0). ^{1}H-NMR spectra were acquired in DMSO-d_6 on a Bruker Avance DPX-400 MHz 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, $HF_{254+366}$, type 60, 0.25 mm, Darmstadt, Germany). The elemental analyses (C, H and N) were performed on Leco CHNS 932 (Leco Cooperation, St. Joseph, MI, USA) analyzer by the Scientific and Technical Research Council of Turkey Instrumental Analysis Laboratories (Ankara, Turkey).

4.1.1. Preparation of 3-benzylidene-4-oxopentanoic acid derivatives (1a-e) (General Procedure A)

An ice-cooled mixture of appropriate aromatic aldehyde (0.02 mol) and levulinic acid (0.03 mol) was saturated with dry hydrogen chloride. Then, the mixture was stirred for 24 h at room temperature. The precipitate which was formed was filtered off and washed with ethyl ether. The crude acids were crystallized from a mixture of ethyl acetate/n-hexane[10].

4.1.2. Preparation of 5-benzylidene-6-methyl-4,5-dihydropyridazine-3(2H)-ones (2a-e) (General Procedure B)

A mixture of pentanoic acid derivatives (1a-e) (0.02 mol) and hydrazine hydrate (0.02 mol) were refluxed for 2h in ethanol (50 ml). Then the mixture was cooled and the crude product which separated was filtered off and crystallized from ethanol.

4.1.3. Preparation of ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3a-e)(General Procedure C)

The appropriate pyridazine-3(2H)-one derivatives (2a-e) (0.02 mol) were added to an ethanolic solution (50 ml) of sodium (0.02 g atom). The mixture was refluxed for 30 min. Then, ethyl bromoacetate (0.02 mol) was added by drops to the cooled solution, which was refluxed for 24 h, and evaporated in vacuo. The residue was collected by filtration, dried and recrystallized from a mixture of ethyl acetate/n-hexane.

4.1.4. Preparation of [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetohydrazide (4a-e) (General Procedure D)

Hydrazine hydrate (0.3 mol) was added to the solution of appropriate ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydro-pyridazine-1(4H)-yl]acetate (3a-e) (0.01 mol) in ethanol (15 ml). The solution was refluxed for 4 h. After cooling, the precipitate which separated was filtered off and recrystallized from ethanol.

4.1.5. Preparation of [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid (5a-e) (General Procedure E)

Sodium hydroxide (0.03 mol) was added to the suspension of appropriate ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydro-pyridazine-1(4H)-yl]acetate (3a-e) (0.01 mol) in ethanol (100 ml). The reaction mixture was refluxed for 4 h and then the ethanol was removed. The residue was dissolved in water (50 ml) and acidified with an aqueous solution of 10% sulfuric acid (pH=1). The precipitate was filtered off, washed with water and recrystallized from methanol/water mixture.

4.1.6. 4-Oxo-3-(4-bromobenzylidene) pentanoic acid (1b)

It was synthesized by the general procedure A using 4-bromobenzaldehyde and levulinic acid, and crystallized from ethyl acetate/n-hexane mixture. Yield 62.89 %, m.p. 216-7°C. UV λ_{max}^{MeOH}; 201 (logε: 4.09), 218 (logε: 4.00 and 280 nm (logε: 4,21): IR (KBr); 3200-2800, 1657, 1229cm^{-1} : ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.45 (s; 3H; -C$\underline{H}_3$), 3.30 (s; 2H; -C$\underline{H}_2$), 7.35-7.60(m; 4H; phenyl prot.) and 7.85(s; 1H; C$\underline{H}$). M.W.: 283.12, Anal. $C_{12}H_{11}BrO_3$, Calcd.: C:50,91, H:4,50. Found: C:51.33, H:3,92.

4.1.7. 5-Benzylidene-6-methyl-4,5-dihydropyridazine - 3(2H)-one (2a)

It was synthesized by the general procedure B using 3-benzylidene-4-oxopentanoic acid and hydrazine hydrate, and crystallized from ethanol. Yield 75.25 %, m.p. 169-70°C (Lit.no. 19: 172°C).

4.1.8. 5-(4-Bromobenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one (2b)

It was synthesized by the general procedure B using 3-(4-bromobenzylidene)-4-oxopentanoic acid and hydrazine hydrate, and crystallized from ethanol. Yield 83.87 %, m.p. 217-8°C. λ_{max}^{MeOH}; 202 (logε: 4.58) and 280 nm (logε: 3.41): IR (KBr); 3200-2850, 1660 cm^{-1} : ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.13 (s; 3H; -C$\underline{H}_3$), 3.84 (s; 2H; -C$\underline{H}_2$), 6.40 (s; 1H; C$\underline{H}$), 7.19 (d; 2H; J=8.3; phenyl, H$^{2'}$, H$^{6'}$), 7.55 (d; 2H; J=8.3;phenyl, H$^{3'}$, H$^{5'}$) and 12.69 ppm (s; ^{1}H; NH). M.W.: 279.14, Anal. $C_{12}H_{11}BrN_2O$, Calcd.: C:51.63, H:3.97, N:10.04. Found: C:51.76, H:4.00, N:10.06.

4.1.9. 5-(4-Chlorobenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one (2c)

It was synthesized by the general procedure B using 3-(4-chlorobenzylidene)-4-oxopentanoic acid and hydrazine hydrate, and crystallized from ethanol. Yield 79.06%, m.p. 203-4°C. (Lit. no.19: 198°C).

4.1.10. 5-(4-Methylbenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one (2d)

It was synthesized by the general procedure B using 3-(4-methylbenzylidene)-4-oxopentanoic acid and hydrazine hydrate, and crystallized from ethanol.

Yield 84.57%, m.p. 210-1°C. (Lit. no.19: 210-2°C).

4.1.11. 5-(5-Methoxybenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one (2e)

It was synthesized by the general procedure B using 3-(4-bromobenzylidene)-4-oxopentanoic acid and crystallized from ethanol. Yield 83.87%, m.p. 217-8°C. UV λ_{max}^{MeOH}; 202 (logε: 4.47) and 278 nm (logε: 4.07): IR (KBr); 3200-2850, 1664 cm^{-1} : ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.14 (s; 3H; -C<u>H</u>$_3$), 3.73 (s; 3H; Ar-OC<u>H</u>$_3$), 3.76 (s; 2H; -C<u>H</u>$_2$), 6.36 (s; 1H; C<u>H</u>), 6.91 (d; 2H; J=8.6 phenyl, H$^{2'}$, H$^{6'}$), 7.13 (d; 2H; J=8.6;phenyl, H$^{3'}$, H$^{5'}$) and 12.66 ppm (s; 1H; NH). M.W.: 279.14, Anal. $C_{13}H_{14}N_2O_2$, Calcd.: C:67.81, H:6.13, N:12.17. Found: C:68.05, H:6.02, N:12.05.

4.1.12. Ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3a)

It was synthesized by the general procedure C using 5-benzylidene-6-methyl-4,5-dihydropyridazine-3(2H)-one (2a) and ethyl bromoacetate, and crystallized from ethyl acetate/n-hexane mixture. Yield 88.71%, m.p. 97-8°C (Lit. no. 10: 90°C).

4.1.13. Ethyl [4-(4-bromobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3b)

It was synthesized by the general procedure C using 5-(4-bromobenzylidene)-6-methyl-4,5-dihydro-pyridazine-3(2H)-one (2b) and ethyl bromoacetate, and crystallized from ethyl acetate/n-hexane mixture. Yield 82.96%, m.p. 139-140°C. UV λ_{max}^{MeOH}; 202 (logε: 4.39) and 292 nm (logε: 3.04): IR (KBr); 1744, 1660, 1208 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.19 (t; 3H; CH_2-C<u>H</u>$_3$), 2.18 (s; 3H; -C<u>H</u>$_3$), 3.89 (s; 2H; -C<u>H</u>$_2$), 4.12-4.16 (q; 2H; -C<u>H</u>$_2$-CH_3), 4.76 (s; 2H; C<u>H</u>$_2$-COO), 6.51 (s; 1H; C<u>H</u>), 7.21 (d; 2H; J=8.3; phenyl, H$^{2'}$, H$^{6'}$) and 7.56 ppm (d; 2H; J=8.3; phenyl, H$^{3'}$, H$^{5'}$). M.W.: 365.23, Anal. $C_{16}H_{17}BrN_2O_3$, Calcd.: C:52.62, H:4.69, N:7.67. Found: C:53.00, H:5.08, N:7.55.

4.1.14. Ethyl [4-(4-chlorobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3c)

It was synthesized by the general procedure C using 5-(4-chlorobenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one (2c) and ethyl bromoacetate, and crystallized from ethyl acetate/n-hexane mixture. Yield 82.96%, m.p. 139-140°C. UV λ_{max}^{MeOH}; 202 (logε: 4.55) and 289 nm (logε: 3.47): IR (KBr); 1744, 1662, 1209 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.19 (t; 3H; CH_2-C<u>H</u>$_3$), 2.19 (s; 3H; -C<u>H</u>$_3$), 3.91 (s; 2H; -C<u>H</u>$_2$), 4.10-4.16 (q; 2H; -CH_2-C<u>H</u>$_3$), 4.76 (s; 2H; C<u>H</u>$_2$-COO), 6.50 (s; 1H; C<u>H</u>), 7.13 (d; 2H; J=8.3; phenyl, H$^{2'}$, H$^{6'}$) and 7.27 ppm (d; 2H; J=8.3; phenyl, H$^{3'}$, H$^{5'}$). M.W.:320.78, Anal. $C_{16}H_{17}ClN_2O_3$, Calcd.: C:59.91, H:5.34, N:8.73. Found: C:59.90, H:5.34, N:8.42.

4.1.15. Ethyl [4-(4-methylbenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3d)

It was synthesized by the general procedure C using 5-(4-methylbenzylidene)-6-methyl-4,5-dihydro-pyridazine-3(2H)-one (2d) and ethyl bromoacetate, and crystallized from ethyl acetate/n-hexane mixture. Yield 86.91%, m.p. 130-1°C (lit. no. 10:130°C).

4.1.16. Ethyl [4-(4-methoxybenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3e)

It was synthesized by the general procedure C using 5-(5-methoxybenzylidene)-6-methyl-4,5-dihydropyridazine-3(2H)-one (2e) and ethyl bromoacetate, and crystallized from ethyl acetate/n-hexane mixture. Yield 79.98%, m.p. 103-5°C. UV λ_{max}^{MeOH}; 202 (logε: 4.53) and 284 nm (logε: 4.14): IR (KBr); 1747, 1667, 1209 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.19 (t; 3H; CH_2-C<u>H</u>$_3$), 2.19 (s; 3H; -C<u>H</u>$_3$), 3.74 (s; 3H; Ar-O-C<u>H</u>$_3$)3.82 (s; 2H; -C<u>H</u>$_2$-), 4.10-4.15 (q; 2H; -C<u>H</u>$_2$-CH_3), 4.76 (s; 2H; C<u>H</u>$_2$-COO), 6.47 (s; 1H; CH), 6.93 (d; 2H; J=8.3; phenyl, H$^{2'}$, H$^{6'}$) and 7.15 ppm (d; 2H; J=8.3; phenyl, H$^{3'}$, H$^{5'}$). M.W.:316.352, Anal. $C_{17}H_{20}N_2O_4$, Calcd.: C:64.54, H:6.37, N:8.86. Found: C:65.04, H:5.74, N:8.79.

4.1.17. [4-Benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl] acetohydrazide (4a)

It was synthesized by the general procedure D using ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3a) and hydrazine hydrate, and crystallized from ethanol. Yield 72.06%, m.p. 165-6°C (Lit. no. 10:167°C).

4.1.18. [4-(4-Bromobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl] acetohydrazide (4b)

It was synthesized by the general procedure D using ethyl [4-(4-bromobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3b) and hydrazine hydrate, and crystallized from ethanol. Yield 64.95%, m.p. 205-6°C. UV $^{MeOH.}_{\lambda max}$: 202 (logε: 4.59) and 292 nm (logε: 3.51): IR (KBr); 3293, 3210, 1663. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.16 (3H; s; C$\underline{H}_3$), 3.87 (2H; s; C$\underline{H}_2$), 4.37 (2H; s; N$\underline{H}_2$), 4.55 (2H; s; C$\underline{H}_2$CONHNH$_2$), 6.47 (1H; s; C=C$\underline{H}$), 7.21 (d; 2H; J=8,2; phenyl, H$^{2'}$, H$^{6'}$), 7.56 (d; 2H; J=8,2; phenyl, H$^{3'}$, H$^{5'}$) and 9.25 ppm (1H; s; NH). M.W.:351.20, Anal. $C_{14}H_{15}BrN_4O_2$, Calcd.: C:47.88, H:4.30, N:15.95. Found: C:47.79, H:4.40, N:15.39.

4.1.19. [4-(4-Chlorobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetohydrazide (4c)

It was synthesized by the general procedure D using ethyl [4-(4-chlorobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3c) and hydrazine hydrate, and crystallized from ethanol. Yield 72.60%, m.p. 196-7°C. UV $^{MeOH.}_{\lambda max}$: 203 (logε: 4.55) and 291 nm (logε: 3.61): IR (KBr); 3306, 3253, 1662. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.17 (3H; s; C$\underline{H}_3$), 3.89 (2H; s; C$\underline{H}_2$), 4.24 (2H; s; N$\underline{H}_2$), 4.55 (2H; s; C$\underline{H}_2$CONHNH$_2$), 6.47 (1H; s; C=C$\underline{H}$), 7.27 (d; 2H; J=8,4; phenyl, H$^{2'}$, H$^{6'}$), 7.43 (d; 2H; J=8,4; phenyl, H$^{3'}$, H$^{5'}$) and 9.21 ppm (1H; s; N$\underline{H}$). M.W.:306.747, Anal. $C_{14}H_{15}ClN_4O_2$, Calcd.: C:54.82, H:4.93, N:18.26. Found: C:54.37, H:4.40, N:17.90.

4.1.20. [4-(4-Methylbenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl] acetohydrazide (4d)

It was synthesized by the general procedure D using ethyl [4-(4-methylbenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3d) and hydrazine hydrate, and crystallized from ethanol. Yield 73.42%, m.p. 193-4°C (Lit. no. 10: 190°C).

4.1.21. [4-(4-Methoxybenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl] acetohydrazide (4e)

It was synthesized by the general procedure D using ethyl [4-(4-methoxybenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3e) and hydrazine hydrate, and crystallized from ethanol. Yield 63.70%, m.p. 172-3°C. UV $^{MeOH.}_{\lambda max}$: 202 (logε: 4.67) and 284 nm (logε: 3.79): IR (KBr); 3299, 3206, 1661. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.17 (3H; s; C$\underline{H}_3$), 3.74 (3H; s; Ar-OC$\underline{H}_3$), 3.80 (2H; s; C$\underline{H}_2$), 4.43 (2H; s; N$\underline{H}_2$), 4.54 (2H; s; C$\underline{H}_2$CONHNH$_2$), 6.42 (1H; s; C=C$\underline{H}$), 6.93 (d; 2H; J=8,6; phenyl, H$^{2'}$, H$^{6'}$), 7.15 (d; 2H; J=8,6; phenyl, H$^{3'}$, H$^{5'}$) and 9.24 ppm (1H; s; N$\underline{H}$). M.W.:302.32, Anal. $C_{15}H_{18}N_4O_3$, Calcd.: C:59.59, H:6.00, N:18.53. Found: C:59.28, H:6.14, N:18.23.

4.1.22. [4-Benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid (5a)

It was synthesized by the general procedure E using ethyl [4-benzylidene-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3a) and sodium hydroxide, and crystallized from methanol/water mixture. Yield 70.54%, m.p. 210-1°C (lit. no. 12: 210°C)

4.1.23. [4-(4-Bromobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid (5b)

It was synthesized by the general procedure E using ethyl [4-(4-bromobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3b) and sodium hydroxide, and crystallized from methanol/water mixture. Yield 62.90%, m.p. 201-2°C. UV $^{MeOH.}_{\lambda max}$: 202 (logε: 4.46) and 294 nm (logε: 3.44). IR (KBr); 3100-2750, 1764, 1639, 1207. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.18 (s; 3H; C$\underline{H}_3$), 3.88 (s; 2H; -C$\underline{H}_2$), 4.67 (s; 2H; C$\underline{H}_2$-COO-), 6.49 (s; 1H; C$\underline{H}$), 7.21 (d; 2H; J=8,3; phenyl, H$^{2'}$, H$^{6'}$), 7.56 (d; 2H; J=8,3; phenyl, H$^{3'}$, H$^{5'}$)

and 12.35 ppm (s; 1H; COOH). M.W.:337.17, Anal. $C_{14}H_{13}BrN_2O_3$, Calcd.: C:49.87, H:3.89, N:8.31. Found: C:50.00, H:3.74, N:8.40.

4.1.24. [4-(4-Chlorobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid (5c)

It was synthesized by the general procedure E using ethyl [4-(4-chlorobenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3c) and sodium hydroxide, and crystallized from methanol/water mixture. Yield 77.74%, m.p. 184-5°C. UV λ_{max}^{MeOH}; 203 (logε: 4.46) and 294 nm (logε: 3.46). IR (KBr); 3100-2750, 1730, 1638, 1209. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.18 (s; 3H; C$\underline{H}_3$), 3.82 (s; 2H; -C$\underline{H}_2$), 4.67 (s; 2H; C$\underline{H}_2$-COO-), 6.48 (s; 1H; C$\underline{H}$), 7.27 (d; 2H; J=8,3; phenyl, H$^{2'}$, H$^{6'}$), 7.43 (d; 2H; J=8,3; phenyl, H$^{3'}$, H$^{5'}$) and 12.78 ppm (s; 1H; COO$\underline{H}$). M.W.:292.17, Anal. $C_{14}H_{13}ClN_2O_3$, Calcd.: C:57.44, H:4.48, N:9.57. Found: C:57.24, H:4.16, N:9.29.

4.1.25. [4-(4-Methylbenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid (5d)

It was synthesized by the general procedure E using ethyl [4-(4-methylbenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3d) and sodium hydroxide, and crystallized from methanol/water mixture. Yield 79.77%, m.p. 173-4°C (Lit.no. 12: 172°C).

4.1.26. [4-(4-Methoxybenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetic acid (5e)

It was synthesized by the general procedure E using ethyl [4-(4-methoxybenzylidene)-3-methyl-6-oxo-5,6-dihydropyridazine-1(4H)-yl]acetate (3e) and sodium hydroxide, and crystallized from methanol/water mixture. Yield 68.75%, m.p. 174-5°C. UV λ_{max}^{MeOH}; 202 (logε: 4.46) and 284 nm (logε: 3.63). IR (KBr); 3100-2750, 1729, 1639, 1209. ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.19 (s; 3H; C$\underline{H}_3$), 3.74 (s; 3H; Ar-OC$\underline{H}_3$), 3.81 (s; 2H; -C$\underline{H}_2$-), 4.66 (s; 2H; C$\underline{H}_2$-COO-), 6.45 (s; 1H; C$\underline{H}$), 6,93 (d; 2H; J=8,5; phenyl, H$^{2'}$, H$^{6'}$), 7.17 (d; 2H; J=8,5; phenyl, H$^{3'}$, H$^{5'}$) and 12.66 ppm (s; 1H; COO$\underline{H}$). M.W.:288.29, Anal. $C_{15}H_{16}N_2O_4$, Calcd.: C:62.49, H:5.59, N:9.72. Found: C:62.89, H:5.17, N:9.75.

5.2. Pharmacology

5.2.1. Animals

Swiss albino mice of 20-25 g supplied from Marmara University, Experimental Research and Animal Laboratory were used for examining the anticonvulsant activities of the compounds. All experiments were carried out with humane methods, and approval of Marmara University Ethical Committee for Experimental Animals was obtained before the experiments. 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.

5.2.2. 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. Pentylenetetrazole (PTZ) (Sigma) freshly dissolved in 0.9% NaCl solution was administered intraperitoneally at a dose of 75 mg/kg.

5.2.3. Pentylenetetrazole (PTZ)-induced seizures test

The anticonvulsant activity was performed by PTZ seizure model. PTZ is a GABAergic non-competitive antagonist that does not interact directly with GABA receptors, but blocks the GABA-mediated Cl-influx. The intraperitoneal (i.p.) injection of PTZ in mice causes behavioral seizures. Either the test compounds and the vehicle carboxymethyl cellulose (CMC) or the reference drugs, DFH and ETX, 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. 1 hour after oral administration of compounds, PTZ was injected i.p. Immediately after injection, the mice were individually placed in glass boxes, which had a transparent front wall. The behavior displayed by each animal was re-

corded for 30 min in a quiet laboratory. These recordings were subsequently used to evaluate the severity of the seizures.

The score used by Finn and Gee[20] and Medina et al.[21] was modified and used for measuring the seizure severity. This modified scoring scale is as follows:

0, No abnormal behavior;
1, First myoclonic jerk (sudden muscle jerk, sometimes accompanied by tail movements and head twitch);
2, Violent myoclonic twitches;
3, Running, bouncing clonus (whole body clonus, with or without loss of righting reflexes);
4, Tonic generalized extension (extreme rigidity with fore and hindlimbs extended caudally).

Each animal received a final score that corresponded to the most severe seizure it presented during the test. The latency of the most severe seizure and its grade were determined to evaluate the anticonvulsant activities of the compounds.

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