NWSA-Physical Sciences
ISSN: 1306-3111/1308-7304
NWSA ID: 2015.10.2.3A0068

Status : Original Study
Received: January 2015
Accepted: April 2015

E-Journal of New World Sciences Academy

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http://dx.doi.org/10.12739/NWSA.2015.10.2.3A0068

THE EFFECTS OF 5,5'-BUTANE-1,4-DIYLBIS[4-ALLYL-2-({4-[3-(TRIFLUOROMETHYL)PHENYL]PIPERAZIN-1-YL}METHYL)- 2,4-DIHYDRO-3H-1,2,4-TRIAZOLE-3-THIONE] COMPOUND ON MDA LEVEL AND VITAMINS IN SERUM, LIVER AND KIDNEY OF RATS

ABSTRACT

The 1,2,4-triazole along with its derivatives were reported to exhibit various pharmacological activities [1]. 1,2,4-triazole moieties have been incorporated into a wide variety of therapeutically interesting drug candidates, including anti-inflammatories, CNS stimulants, sedatives, anti-anxiety compounds, antimicrobial agents, as well as anti-mycotic ones such as fluconazole, intraconazole andvoriconazole [2]. As previously reported, Aminomethyl derivatives were synthesized. In this study, the effects of newly synthesized bis-1,2,4-triazole containing aminomethyl derivatives on the level of malondialdehyde(MDA)and antioxidant vitamins (A,E,C) of the serum, liver and kidney of rats have been investigated. In addition, level of malondialdehyde (MDA) and vitamins (A,E,C) has been determined by HPLC [3]. Most of the compounds showed satisfactory performance when compared to the control group [4].

Keywords: 1,2,4-Triazole, Aminomethyl Derivative, Vitamin, Serum, Kidney, Liver, Rat

5,5'-BÜTAN-1,4-DIILBIS[4-ALLIL-2-({4-[3(TRIFLOROMETIL)FENIL]PIPERAZIN-1-IL}METIL)-2,4-DIHIDRO-3H-1,2,4-TRIAZOL-3-TIYON BİLEŞİĞİNİN FARELERİN BÖBREK, KARACİĞER VE SERUMLARINDAKİ MDA VE VİTAMİN DÜZEYLERİNE ETKİSİ

ÖZET

1,2,4-triazol türevleri çeşitli farmakolojik aktivite gösterdikleri bilinmektedir. flukonazol, intraconazole, vorikonazol gibi 1,2,4-triazol türevleri içeren kısımlar çeşitli uyuşturucu ve terapötik ilaçlar da dahil olmak üzere anti-enflamatuar, CNS uyarıcılar, yatıştırıcılar, sakinleştirici maddeler, antibiyotik antimikotik etkilere sahiptir. Kullanılan aminometil türevi daha önce bildirildiği gibi yeniden sentezlendi. Bu çalışmada, yeni sentezlenmiş bis-1,2,4-triazol içeren aminometil türevi bileşik rat serum, karaciğer ve böbrek dokularında malondialdehit(MDA)ve antioksidan vitamin (A,E,C)düzeyleri incelendi. Malondialdehit düzeyi (MDA) ve vitaminler (A,E,C) HPLC cihazı kullanılarak belirlendi. L bileşiği kontrol ile karşılaştırıldığında iyi aktivite gösterdiği belirlendi.

Anahtar Kelimeler: 1,2,4-Triazol, Aminometil Türevi, Vitamin, Serum, Karaciğer, Böbrek, Fare

1. INTRODUCTION (GİRİŞ)

In the last few decades, the chemistry of the 1,2,4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological significance. 1,2,4-triazole moieties have been incorporated into a wide range of therapeutically interesting drug candidates, including anti-inflammatory, CNS stimulants, sedatives, anti-anxiety compounds, antimicrobial agents [5, 6 and 7] and anti-mycotic ones such asfluconazole, intraconazole andvoriconazole [8 and 9]. There are marketed drugs containing the 1,2,4-triazolegroup, *e.g.*, triazolam, alprazolam, etizolam and furacylin [10].

The purpose of this study was to assess the MDA level and antioxidant vitamins i.e. A,C and E vitamins in the liver, kidney and serum of experimental rats. It also aims to control group rats and compare these with different bis-1,2,4-triazole containing aminomethyl derivatives according to the control [11].

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

In everyday life, the use of biologically active compounds is limited due to the challenges associated with administration, high risk of toxicity, onset of drug resistance, unfavorable side effects. Today, many of the compounds used as therapeutic agents contain a 5-membered heterocyclic ring such as triazole, thiadiazole and oxidiazole. As 5-membered heterocyclic compounds have various applications, their synthesis has become increasingly important in recent years. The most important field of use for these 5-membered heterocyclic compounds is chemotherapeutic agents and the most biologically active isomers include 1,2,4-triazole, 1, 3, 4-thiadiazole,as well as 1, 3, 4-oxidiazole derivatives [12]. The effects of compounds on tissues and serum vitamin A,E,C levels and MDA values in test animals have been assessed in vivo.

3. EXPERIMENTAL METHOD (DENEYSEL ÇALIŞMA)

3.1. Animal Treatments (Hayvan Uygulamaları)

The following experiments were approved by the Ethical Committee for care and use of laboratory animals in Fırat University [13]. Experiments were performed 6-8 times per week on male Long Evans rats weighing between 200-240g. The rats were allowed unrestricted access to food and water. Room temperature was maintained at 22±1°C with a 12-hour's light-dark cycle [10]. The animals were randomly divided into two groups (the Control and L groups), with each group containing five rats.Bis-1,2,4-triazole containing amino methyl derivatives was diluted with corn oil in such a manner that its amount would be below 10% as dimethyl sulfoxide (DMSO) dissolved [14 and 15]. Animals were divided into one "control group" and an "implementation group", including five for each. Corn oil-diluted DMSO was injected to the control group. 0.5ml DMSO, including 25 mg/kg was injected subcutaneously to derivates groups for thirty days with a three-day interval during the test [10, 13 and 16]. These procedures continued for thirty days after which time each experimental rat was anesthetized and decapitated then blood samples were collected in tubes and stored in -20°C prior to the biochemical analysis. The blood samples centrifuged at 4500 x g for ten minutes and the serum was separated to get tested for vitamins and MDA. In addition, the livers and kidneys were removed for a vitamins and MDA analysis. 300mg liver and 300mg kidney tissue samples were homogenized in 3ml acetonitrile/methanol/isopropyl alcohol-containing (2:1:1, v/v/v) tubes then the samples were vortexed for 30s and centrifuged at 6000xg

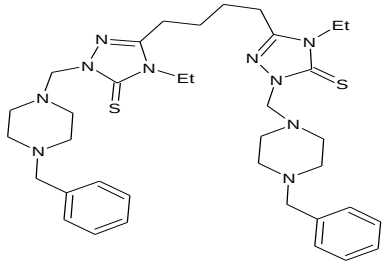
for 10 minutes. Supernatants were transferred to auto-sampler vials of the HPLC instrument [13 and 17].

3.2. Chemicals (Kimyasallar)

All solvents were of analytical-grade reagents. Bis-1,2,4-triazole, containing the amino methyl derivatives used in the applications, were synthesized and characterized by Koparir et al. [18]. The structure of derivatives and IUPAC Nomenclature are below [15 and 18](Fig. 1 and Tab.1).

Table 1. IUPAC nomenclature of compound L
(Tablo 1. L bileşiğinin adlandırılması)

L	5,5'-bütan-1,4-diilbis[4-allil-2-({4-[3-(triflorometil)fenil]piperazin-1-il}metil)-2,4-dihidro-3H-1,2,4-triazol-3-tiyon



L

Figure 1. Chemical structure of L compound
(Şekil 1. L bileşiğinin kimyasal formülü)

3.3. Analytical Methods (Analitik Metotlar)

The liquid chromatographic system (Shimadzu) consisted of twoLC-20AD pumps, aDGU-20A5 degasser, a Sil20A auto-sampler, a CTO-10As VP column oven, an SPD-M20ADAD system, and an RF-10AXL Fld system. The two detectors were connected in series [19].

3.3.1. Determination of Tissues Vitamin C and MDA Levels (Dokularda C Vitaminive MDA Düzeylerinin Belirlenmesi)

0.3 grams of liver and kidney tissue samples were taken and homogenized in a mixture of 1.5ml of $HClO_4$ (0.5 M) and 1.5 ml distilled water. Afterwards, tissue samples were centrifuged at 4500 rpm for 25 minutes [20]. After the mixture had centrifuged, 20 µl of samples, taken carefully from supernatants, were injected into the HPLC system. The detection was performed at 254 nm for vitamin C and MDA. Finally, results were calculated as (µg/g) MDA tissue and as µg/g for C vitamin [21].

3.3.2. Determination of Tissues Vitamin A and E Levels (Dokularda A ve E Vitamini Düzeylerinin Belirlenmesi)

0.3 grams of liver and kidney tissue samples were obtained then 4 ml of ethyl alcohol (containing 1%H_2SO_4) were added for precipitated proteins. Following vortexing, the samples were centrifuged at 4500 rpm for 25 minutes. After the mixture centrifuged, 0.3 ml of n-hexane was added and the tubes were vortexed and centrifuged one more. At the end of the centrifugation process, the hexane mixture was taken carefully to a glass tube, and 0.3 ml of n-hexane was added and centrifuged again. The Hexane mixture was caused to evaporate with nitrogen flow and the residue was made to dissolve in 100 µL of methanol and 20 µl of the sample were injected into the HPLC system

[11].The detection was performed at 326 nm for vitamin A, and 296 nm for vitamin E. The results of the analysis were expressed as µg/g [13 and 21].

3.3.3. Determination of Serum Vitamin C and MDA Levels (Serumda C Vitamini ve MDA Düzeylerinin Belirlenmesi)

A volume of 0.3ml of serum sample was taken then 0.3 ml of 0.5M $HClO_4$ was added for precipitated proteins. This mixture was then vortexed and pure water was added to the total 1ml volume. After 15 minutes, the mixture centrifuged (2500rpm/min) and then 20µl of samples were carefully taken from above supernatants and injected on the HPLC. The detection was performed at 254nm for vitamin C and MDA. Finally, results were calculated as µg/ml for MDA and C vitamin [22].

3.3.4. Determination of Serum Vitamin A and E Levels (Serumda A ve E Vitamini Düzeylerinin Belirlenmesi)

0.3 ml of serum samples was taken and another 0.3ml of ethyl alcohol (containing 1%H_2SO_4) was added for precipitated proteins. After vortexing, the samples centrifuged at 2500rpm for 5min [20]. Following the centrifugation phase, 250µl of n-hexane were added and the tubes were vortexed and centrifuged once again. At the end of centrifugation, the hexane mixture was taken carefully to a glass tube and 250µl of n-hexane were added and centrifuged one more time. The Hexane formula was caused to evaporate with nitrogen flow, and the residue was made to dissolve in a 100µl of methanol and 20µl of sample were injected into the HPLC system [11]. The detection was performed at 326nm for vitamin A, and 296nm for vitamin E. In the end, the results were calculated as µg/mL for A and E vitamins [11, 23 and 24].

3.4. Statistical Analysis (İstatistiksel Analiz)

For a statistical analysis, the SPSS 15.0 software program was used. The experimental results were reported as mean ± S.D, while the comparison between the experimental groups and the control one was drawn using ANOVA and LSD tests [11].

4. FINDINGS (BULGULAR)

4.1. Tissues Vitamin A, E, C and MDA Levels (Dokularda Vitamin A, E, C ve MDA Düzeyleri)

All results of liver tissues are presented in Table 2.

Table 2. The contents of vitamins (A,C,E) and MDA levels in the liver tissues of experimental and control group rats
(Tablo2. Deneyve control grubu rat karaciğer dokularında Vitamin A,E,C ve MDA Düzeyleri)

Parameters	Groups	
	Control	L
C Vitamin(µg/g)	14,20±2,27^{ab}	19,86±1,62^{a}
MDA(µg/g)	5,26±0,60^{b}	3,40±0,36^{b}
A Vitamin(µg/g)	0,21±0,01^{a}	0,20±0,01^{a}
E Vitamin(µg/g)	0,62±0,08^{a}	0,65±0,12^{a}

a-b Mean values with different superscripts on the same row are significantly different [25].*P<0.05; Nİ: unimportant P>0.05: Statistical values

All kidney tissues results are shown in Table 3.

Table 3. The contents of vitamins (A,C,E) and MDA levels in the kidney tissues of the rats belonging to both the experimental and control groups. Results are given as mean ± standard deviation (SD), n=5
(Tablo 3. Deney ve control grubu böbrek dokularında Vitamin A,E,C ve MDA Düzeyleri)

Parameters	Groups			
	Control		L	-P-
C Vitamin(µg/g)	10,40±2,16[a]	15,06±0,82[ab]	*	
MDA(µg /g)	4,00±0,42[a]	2,86±0,79[b]	*	
A Vitamin(µg/g)	0,18±0,01[ab]	0,26±0,02[a]	**	
E Vitamin(µg/g)	0,68±0,04[a]	0,69±0,04[a]	Nİ	

a-b Mean values with different superscripts on the same row are significantly different [25].*P<0.05; Nİ: unimportant P>0.05: Statistical values

4.2. Serum Samples Vitamin A,E,C and MDA levels (Serumda Vitamin A,E,Cve MDA Düzeyleri)

All results are presented in Table 4.

Table 4.The contents of vitamins (A,C,E) and MDA levels in the kidney tissues of the rats belonging to both the experimental and control groups. Results are given as mean ± standard deviation (SD), n=5.
(Tablo 4.Deney ve kontrol grubu böbrek dokularında vitamin A,E,C ve MDA düzeyleri)

Parameters (µg/ml)	Groups	
	Control	L1
C Vitamin	3,80±0,22[a]	4,18±0,19[a]
MDA	0,54±0,02[a]	0,30±0,05[b]
A Vitamin	0,56±0,02[a]	0.62±0,03[a]
E Vitamin	3,39±0,26[a]	3,66±0,28[ab]

a-b Mean values with different superscripts on the same row are significantly different.*P<0.05; Nİ: unimportant P>0.05: Statistical values

5. CONCLUSIONS AND RECOMMENDATIONS (SONUÇLAR VE ÖNERİLER)

In this study, MDA and antioxidant vitamins were determined in the serum and liver and kidney tissues obtained from healthy rat tissues by HPLC. Vitamin C level increased in liver tissues in the L-treated compound, compared to the "control group", while vitamin A and E levels were similar in the L-treated group, compared to the "control group" [12 and 19]. MDA levels decreased in the L-treated group and statistical differences were shown when compared to the "control group" [19 and 25]. On the other hand, vitamin C level increased in the kidney tissues in the L-treated group, compared to the "control group" [15, 25 and 26]. The level of vitamin A increased in the L-treated group, while Vitamin E levels were similar in the L-treated group, compared to the 'control group' [25, 26 and 27]. The MDA level decreased in the L-treated groups, compared to the "control group" [25 and 28]. In the serum samples, vitamin C level increased in the L-treated group, compared to the "control group" [25 and 27]. Vitamin A and E levels increased with similar values in the L-treated group, compared to the "control group" [25 and 26]. Besides, the MDA levels decreased in the L-treated groups, compared to the "control group" and e statistical differences were shown within the control group. It was concluded that based on all synthesized derivatives, compounds L with trifluoromethylphenyl-piperazine moiety exhibited the best biological activities, which far exceeded the performance of the control group. So, it was concluded that the presence of trifluoromethylphenyl-piperazine moiety was found to be essential for their high biological

activity [27]. In conclusion, the result study reports the successful synthesis and biological activity of newbis-1,2,4-triazole (5,5'-bütan-1,4-diilbis[4-allil-2-({4-[3(trifloro metil)fenil]piperazin-1-il}metil)-2,4-dihidro-3H-1,2,4-triazol-3-tiyon),containing aminomethyl compound [28]. The antioxidant activity revealed that the tested compound has good antioxidant performance, which may be due to the presence of trifluoromethylphenyl-piperazine moiety, in addition to ally group [28]. Hence, it is concluded that there is ample scope for further study [28].

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