Research Article

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Evaluation of Antioxidant Activity of 1, 2, 4-Triazole Derivatives With Morpholine Moiety

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ABSTRACT

The aim of the work was to study the antioxidant activity of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thioles and their alkyl derivatives in vitro using the method of non-enzymatic initiation of lipid peroxidation. Among the 45 compounds studied, 8 showed varying degrees of AOA. The most pronounced AOA has 4-amino-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiol. It was found that in most cases the introduction in alkyl-derivatives of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thioles free amino group or phenyl substituent to the N4 atom of the 1,2,4-triazole nucleus, accompanied with increasing of AOA for test substances. In almost all cases, the presence on the N4 atom of 1,2,4-triazole nucleus methyl or ethyl groups has the opposite effect, even in comparison with control groups.

Keywords: 1,2,4-triazole, antioxidant activity, in vitro method

1. Introduction

Antioxidants belong to the class of natural or synthetic substances that can slow down or stop the oxidation of mainly organic compounds [1]. They have a wide range of applications, which extends not only to medicine and pharmacy but also to the agricultural industry [2]. Excessive amounts of reactive oxygen poses a great threat to life and health of the human body due to the activation of free radical processes, and as a consequence of the formation of oxidative stress [3].

The world scientific community is actively conducting research for creating and studying of new molecules with antioxidant properties [4, 5]. Analyzing the array of scientific data, a special attention should be noted to the derivatives of 1,2,4-triazole [6, 7]. This nitrogen-containing, heterocyclic system is increasingly attracting the attention of scientists, which based at their level of a wide and pronounced range of biological and pharmacological [8-14] actions and low toxicity parameters [7, 11, 15]. 1,2,4-Triazole derivatives are quite convenient agents in terms of "fine" organic synthesis [16-18] and in terms of relative safety of reagents [19, 20]. Therefore, based on the results of synthetic scientific schools [4, 6-11] and our own work [12-15, 18], we considered it appropriate to study the antioxidant activity (AOA) of 4-R-3-(morpholinomethyl)-*4H*-1,2,4-triazole-5-thioles and their alkyl derivatives *in vitro* using the method of non-enzymatic initiation of lipid peroxidation.

2. Material and Methods

2.1 Chemicals and equipment

The initial compounds 4-R-3-(morpholinomethyl)-*4H*-1,2,4-triazole-5-thioles **(2.6-2.10)** and corresponding alkyl-derivatives **(2.21-2.60)** were synthesized by us earlier at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry of the Zaporizhzhia State Medical University (Ukraine) and purified by recrystallization with content of the main component ≥ 98% (Figure 1) [21, 22]. All chemicals, which mentioned in this work, were obtained from UKRORGSYNTEZ Ltd (Kyiv, Ukraine) with documental approving of its purity and quality.

2.2 Biological part

The AOA study was performed *in vitro* according to the guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine using the method of non-enzymatic initiation of lipid peroxidation (LP) [23].

As a model oxidation system in the study of total AOA 4-R-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thioles (**2.6-2.10**) and corresponding alkyl-derivatives (**2.21-2.60**) was used suspension lipoproteins of egg yolk in which the initiation of LP was carried out by using divalent iron ions [24]. The test substances were introduced into the suspension at a concentration of 10^{-3} mol/l.

The intensity of LP processes in the model system was evaluated by the concentration of active products that react with 2-thiobarbituric acid (TBA-AP). The content of TBA-AP was determined after their extraction with butan-1-ol and measured the optical density of the extract against butan-1-ol (λ = 532 nm). The calculation of AOA (%) was performed according to the formula:

$$\text{AOA} = \frac{\mathbf{D}_c - \mathbf{D}_t}{\mathbf{D}_c} \times \mathbf{100}\%,$$

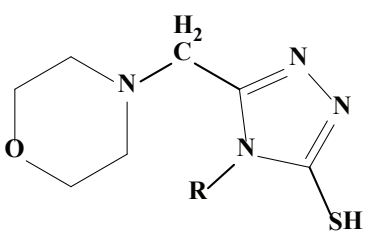
where $\mathbf{D}_c$ - the optical density in the control sample;

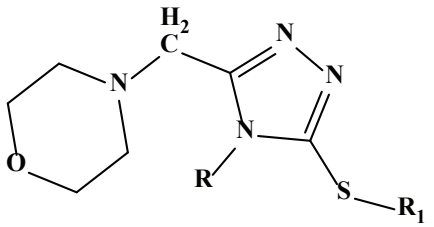
$\mathbf{D}_t$ - the optical density in the test sample.

The significance of intergroup differences according to experimental data was established using Student's t-test. The level of statistical significance of differences in research results is $p < 0.05$ [25, 26].

3. Results and Discussion

As a result of the experiment of determination the AOA of new derivatives of 1,2,4-triazole (Table 1, Figure 2) under conditions of Fe^{2+} - induced lipid peroxidation (LP), it was found that from the **45** compounds **8** (**2.10, 2.24, 2.25, 2.29, 2.30, 2.40, 2.45, 2.55**) in varying degrees of intensity showed the ability to inhibit the generation of free radicals, exceeding reference drug ascorbic acid (AA). Also, the results of the studies allowed to detect substances (**2.22, 2.28, 2.32, 2.36, 2.38, 2.41, 2.42, 2.47, 2.48, 2.57, 2.58)**, which on the contrary lead to an increase the level of TBA-AP.





2.6 R=H;
2.7 R=-CH_3;
2.8 R=-C_2H_5;
2.9 R=-C_6H_5;
2.10 R=-NH_2.

2.21	R=H; R_1=-C_3H_7-n;	**2.41**	R=H; R_1=-C_7H_{15}-n;
2.22	R=-CH_3; R_1=-C_3H_7-n;	**2.42**	R=-CH_3; R_1=-C_7H_{15}-n;
2.23	R=-C_2H_5; R_1=-C_3H_7-n;	**2.43**	R=-C_2H_5; R_1=-C_7H_{15}-n;
2.24	R=-C_6H_5; R_1=-C_3H_7-n;	**2.44**	R=-C_6H_5; R_1=-C_7H_{15}-n;
2.25	R=-NH_2; R_1=-C_3H_7-n;	**2.45**	R=-NH_2; R_1=-C_7H_{15}-n;
2.26	R=H; R_1=-C_4H_9-n;	**2.46**	R=H; R_1=-C_8H_{17}-n;
2.27	R=-CH_3; R_1=-C_4H_9-n;	**2.47**	R=-CH_3; R_1=-C_8H_{17}-n;
2.28	R=-C_2H_5; R_1=-C_4H_9-n;	**2.48**	R=-C_2H_5; R_1=-C_8H_{17}-n;
2.29	R=-C_6H_5; R_1=-C_4H_9-n;	**2.49**	R=-C_6H_5; R_1=-C_8H_{17}-n;
2.30	R=-NH_2; R_1=-C_4H_9-n;	**2.50**	R=-NH_2; R_1=-C_8H_{17}-n;
2.31	R=H; R_1=-C_5H_{11}-n;	**2.51**	R=H; R_1=-C_9H_{19}-n;
2.32	R=-CH_3; R_1=-C_5H_{11}-n;	**2.52**	R=-CH_3; R_1=-C_9H_{19}-n;
2.33	R=-C_2H_5; R_1=-C_5H_{11}-n;	**2.53**	R=-C_2H_5; R_1=-C_9H_{19}-n;
2.34	R=-C_6H_5; R_1=-C_5H_{11}-n;	**2.54**	R=-C_6H_5; R_1=-C_9H_{19}-n;
2.35	R=-NH_2; R_1=-C_5H_{11}-n;	**2.55**	R=-NH_2; R_1=-C_9H_{19}-n;
2.36	R=H; R_1=-C_6H_{13}-n;	**2.56**	R=H; R_1=-$C_{10}H_{21}$-n;
2.37	R=-CH_3; R_1=-C_6H_{13}-n;	**2.57**	R=-CH_3; R_1=-$C_{10}H_{21}$-n;
2.38	R=-C_2H_5; R_1=-C_6H_{13}-n;	**2.58**	R=-C_2H_5; R_1=-$C_{10}H_{21}$-n;
2.39	R=-C_6H_5; R_1=-C_6H_{13}-n;	**2.59**	R=-C_6H_5; R_1=-$C_{10}H_{21}$-n;
2.40	R=-NH_2; R_1=-C_6H_{13}-n;	**2.60**	R=-NH_2; R_1=-$C_{10}H_{21}$-n.

Figure 1. Structural formulas of 4-R-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thioles (**2.6-2.10**) and corresponding alkyl-derivatives (**2.21-2.60**) for which the study of antioxidant activity was performed

Analyzing the data of experimental studies, it was found that the most active among the studied compounds were 4-amino-3-(morpholinomethyl)-*4H*-1,2,4-triazole-5-thiol (**2.10**), which reduces the level of TBA-AP for 42,50% ($p>0.05$) and 3-(morpholinomethyl)-5-(propylthio)-*4H*-1,2,4-triazol-4-amine (**2.25**), which inhibits the formation of the final lipid oxidation products (LOPs) for 41.90% ($p<0.001$) respectively. After analyzing the research data, some regularities were established regarding to the chemical structure and intensity of AOA of named compounds. Thus, almost all studied 4-R-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thioles (**2.6-2.10**) in varying degrees of intensity showed the ability of antioxidant action. Subsequent transition to the corresponding alkyl-derivatives (**2.21-2.60**) ambiguously affects the antioxidant effect of the synthesized substances. It is noted that alkyl-derivatives which containing free amino group on N_4 atom of the 1,2,4-triazole nucleus **(2.25, 2.30, 2.35, 2.40, 2.45, 2.50, 2.55, 2.60)** or phenyl radical **(2.24, 2.29, 2.34, 2.39, 2.44, 2.49, 2.54, 2.59)** in almost cases inhibited the formation of the final product of LOPs at the level from 41.90% ($p<0.001$) to 11.73% ($p<0.01$) and from 38.11% ($p<0.05$) to 10.29% ($p<0.01$), respectively. The increasing quantity of carbons in the hydrocarbon chain of alkyl-derivatives (**2.21-2.60**) moderately reduces the AOA of the compounds, and the introduction of the n-decyl fragment (**2.56-2.60**) significantly reduces named effect in range from -6.74% ($p<0.01$) to 11.73% ($p<0.01$). It was noted, that in almost all cases the presence at the N_4 atom of the nucleus of 1,2,4-triazole methyl (**2.22, 2.32, 2.37, 2.41, 2.47, 2.57**) or ethyl (**2.23, 2.28, 2.37, 2.48, 2.58**) groups causes the opposite effect, and increases increase the level of TBA-AP even comparing to the control groups (Table 1, Figure 2).

To establish the relationship dependence of «structure-AOA» a hypothesis about the absence of differences of obtained results under the conditions by compounds application (**2.6-2.10, 2.21-2.25, 2.26-**

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2.30, 2.31-2.35, 2.36-2.40, 2.41-2.45, 2.46-2.50, 2.51 -2.55, 2.56-2.60, Figure 3-11) was put forward. The validity of the hypothesis about the similarity of the sample data was rejected using Student's t-test. Thus, it was found that the introduction in N_4 position of 1,2,4-triazole thione free amino group (-NH_2, 2.10) leads to a very high ability to reduce the level of TBA-AP ($p < 0.01$) (Figure 3). Considering the calculated statistical differences in compounds **2.21-2.25**, which contain an n-propyl fragment with methyl (**2.22**) and ethyl (**2.23**) substituents at N_4 position of 1,2,4-triazole indicated an absence of AOA. In this case, the introduction of phenyl **(2.24**) substituent or amino group (**2.25**) leads to a significant AOA in comparison with compounds **2.21** and **2.22** (Figure 4). Subsequent introduction of pentyl **2.31-2.35** and hexyl **2.36-2.40** radicals leads to a very pronounced loss of antioxidant action (Figure 6, 7). During considering compounds which includes a heptyl substituent (**2.41-2.45**, Figure 8) AOA was observed with the intensity that is not significantly inferior to the reference drug (Ascorbic acid), while compound **2.45** exceeds the activity of Ascorbic acid and within its confidence interval ($p>0.05$).

The introduction of octyl (**2.47-2.49**), nonyl (**2.51-2.54**) and decyl (**2.57-2.59**) substituents (Figure 9-11) did not show AOA and not exceed the action of Ascorbic acid ($p> 0.05$).

Conclusions

Among the 45 compounds studied, 8 showed varying degrees of AOA. The most pronounced AOA has 4-amino-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thiol (**2.10**), which reduces the level of TBA-AP for 42.50% ($p> 0.05$). It was found that in most cases the introduction in alkyl-derivatives of 4-R-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thioles (**2.21-2.60**) free amino group or phenyl substituent to the N_4 atom of the 1,2,4-triazole nucleus, accompanied with increasing of AOA for test substances. In almost all cases, the presence on the N_4 atom of 1,2,4-triazole nucleus methyl or ethyl groups has the opposite effect, even in comparison with control groups.

Acknowledgements

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Table 1. Antioxidant activity of compounds 2.6-2.10, 2.21-2.60 in vitro with non-enzymatic initiation of LP

Compound	Optical density (λ=532 nm) M±σ (n=7)	AOA, %	Compound	Optical density (λ=532 nm) M±σ (n=7)	AOA, %
Ascorbic acid	0.494±0.00320	27,79	**Ascorbic acid**	0.485±0.0034	30,7
Control group	0.6841±0.0059	0	**Control group**	0.6999±0.0075	0
2.6	0.6491±0.0032	5,12	**2.41**	0.7282±0.0069	-4,04
2.7	0.6026±0.0098	11,91	**2.42**	0.7568±0.0032	-8,13
2.8	0.6142±0.0066	10,22	**2.43**	0.5361±0.0056	23,4
2.9	0.5252±0.0065	23,23	**2.44**	0.5428±0.0058	22,45
2.10	0.3934±0.0050	42,50	**2.45**	0.4232±0.0069	39,53
Ascorbic acid	0.4961±0.0037	28,46	**Ascorbic acid**	0.484±0.0038	32,05
Control group	0.6934±0.0067	0	**Control group**	0.7123±0.0027	0
2.21	0.5804±0.0062	16,3	**2.46**	0.5635±0.0072	20,89
2.22	0.7502±0.0074	-8,19	**2.47**	0.7778±0.0048	-9,2
2.23	0.6857±0.0071	1,11	**2.48**	0.7966±0.0066	-11,83
2.24	0.4291±0.0063	38,12	**2.49**	0.6258±0.0064	12,14
2.25	0.4029±0.0026	41,90	**2.50**	0.5161±0.0057	27,54
Ascorbic acid	0.4665±0.0025	30,18	**Ascorbic acid**	0.4736±0.0048	35,71
Control group	0.6681±0.0055	0	**Control group**	0.7367±0.0069	0
2.26	0.5196±0.0061	22,23	**2.51**	0.6248±0.0051	15,19
2.27	0.5651±0.0073	15,42	**2.52**	0.5664±0.0081	23,12
2.28	0.7433±0.0045	-11,26	**2.53**	0.5182±0.0082	29,66
2.29	0.4525±0.0065	32,27	**2.54**	0.5141±0.0054	30,22
2.30	0.4135±0.0058	38,11	**2.55**	0.4674±0.0054	36,56
Ascorbic acid	0.4816±0.0042	30,66	**Ascorbic acid**	0.4807±0.0051	28,11
Control group	0.6946±0.0076	0	**Control group**	0.6686±0.0053	0
2.31	0.5781±0.0083	16,77	**2.56**	0.6094±0.0063	8,85
2.32	0.737±0.0052	-6,1	**2.57**	0.7137±0.0056	-6,74
2.33	0.611±0.005	12,04	**2.58**	0.676±0.0035	-1,1
2.34	0.5634±0.0041	18,89	**2.59**	0.5998±0.0063	10,29
2.35	0.5332±0.0062	23,23	**2.60**	0.5902±0.006	11,73
Ascorbic acid	0.4863±0.0056	32,93			
Control group	0.7251±0.0051	0			
2.36	0.7307±0.0044	-0,77			
2.37	0.7177±0.0043	1,03			
2.38	0.8073±0.0039	-11,33			
2.39	0.5105±0.0065	29,60			
2.40	0.4839±0.0048	33,27			

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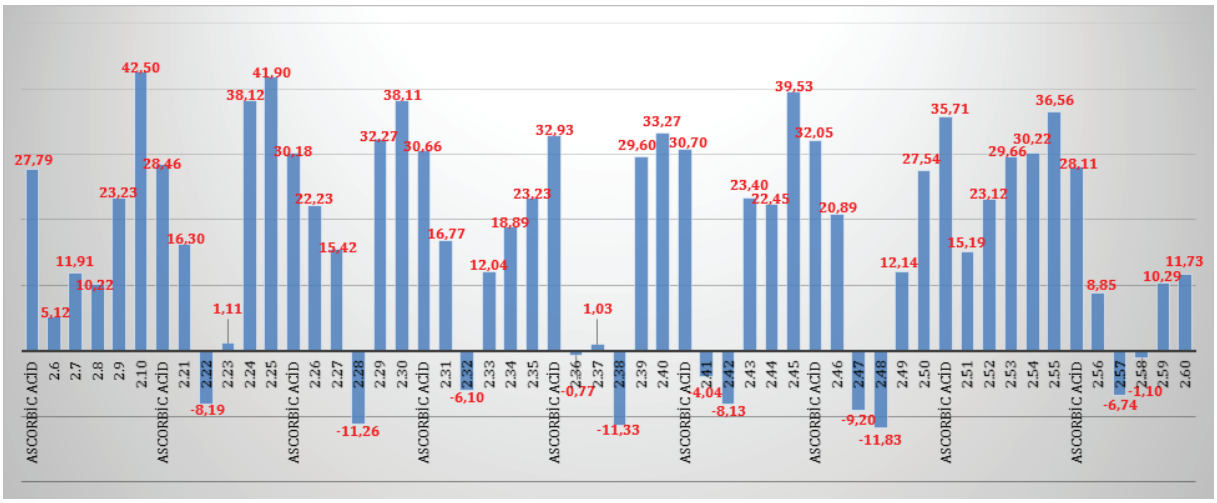

Figure 2. The graphical data of antioxidant activity of 4-R-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thioles **(2.6-2.10)** and corresponding alkyl-derivatives **(2.21-2.60), %.**

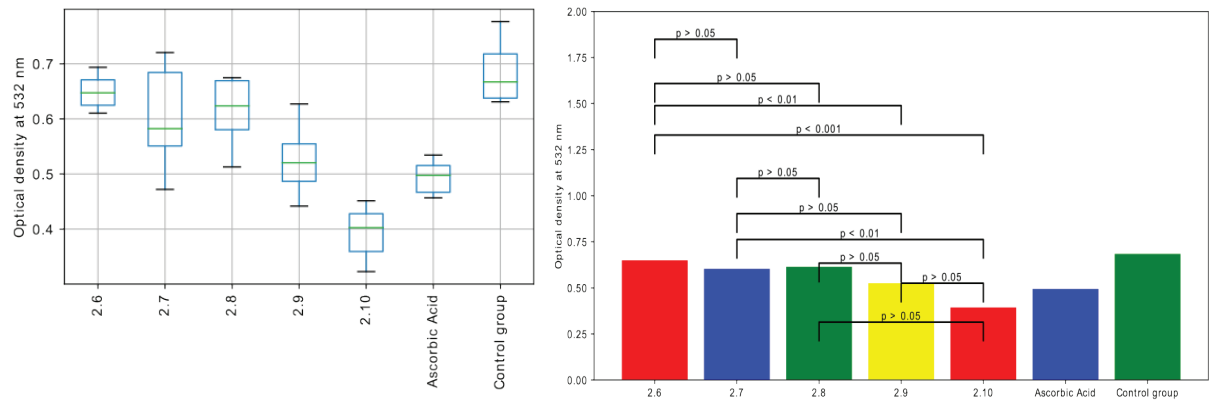

Figure 3. Intergroup and within-group comparisons AOA (Optical density) of 4-R-3-(morpholinomethyl)-4*H*-1,2,4-triazole-5-thioles **(2.6-2.10)**

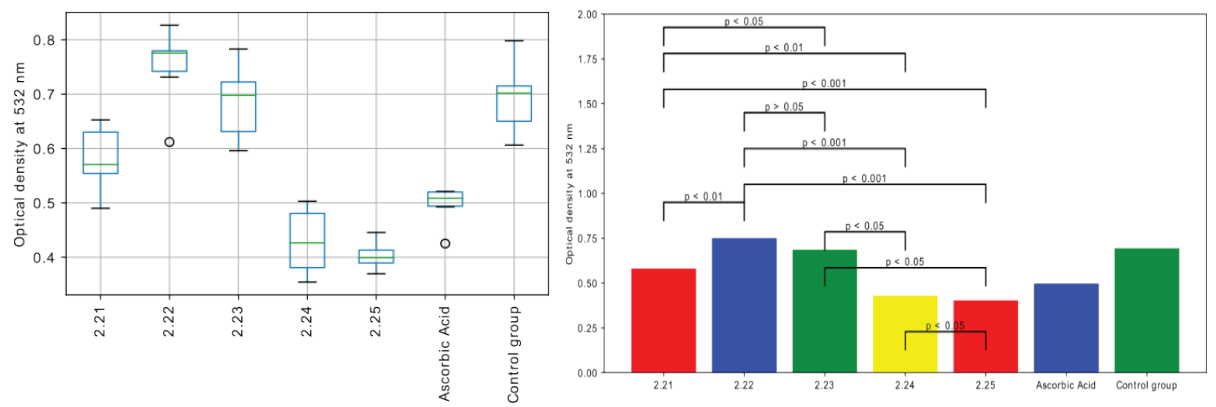

Figure 4. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(propylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine **(2.21-2.25)**

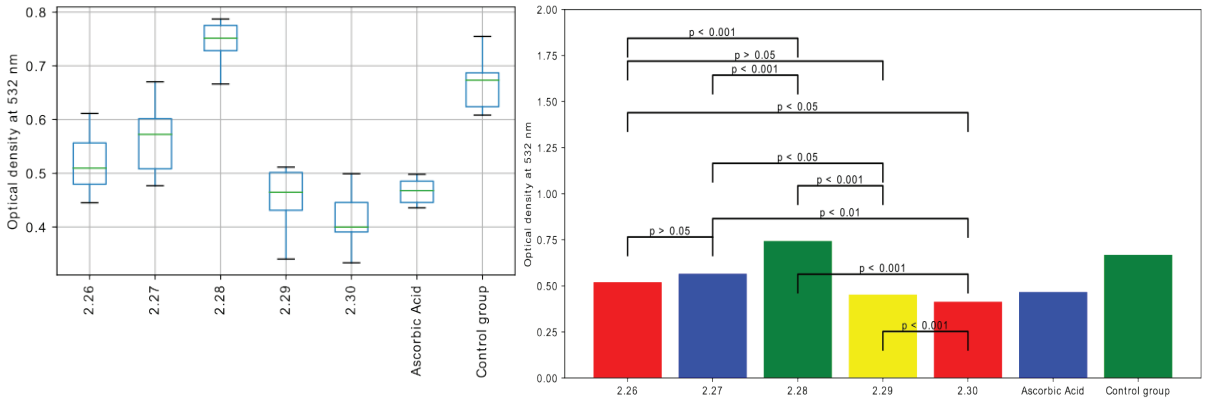

Figure 5. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(butylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine **(2.26-2.30)**

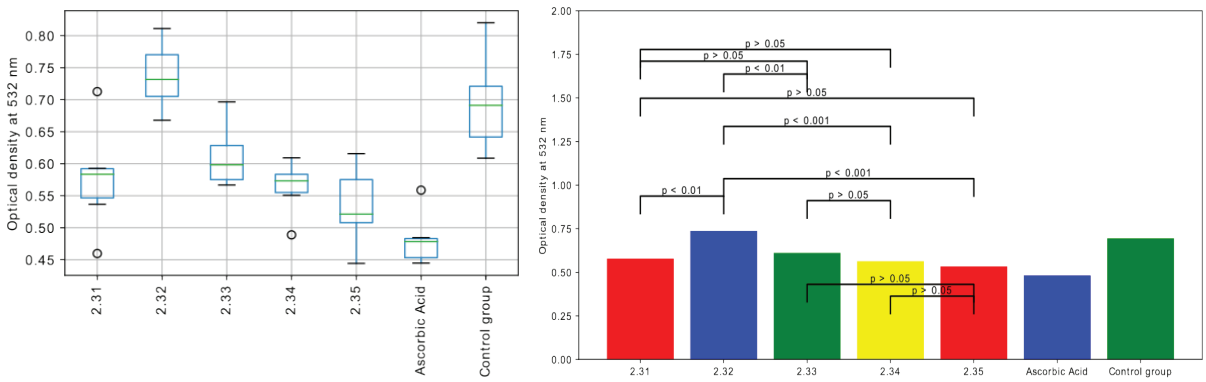

Figure 6. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(pentylthio)-4*H*-1,2,4-triazol-3-yl) methyl) morpholine (**2.31-2.35**)

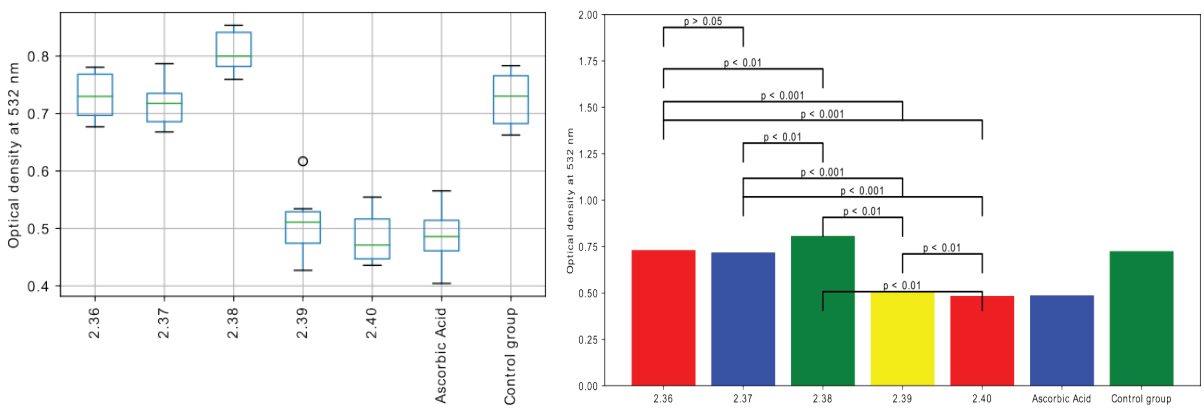

Figure 7. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(hexylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine (**2.36-2.40**)

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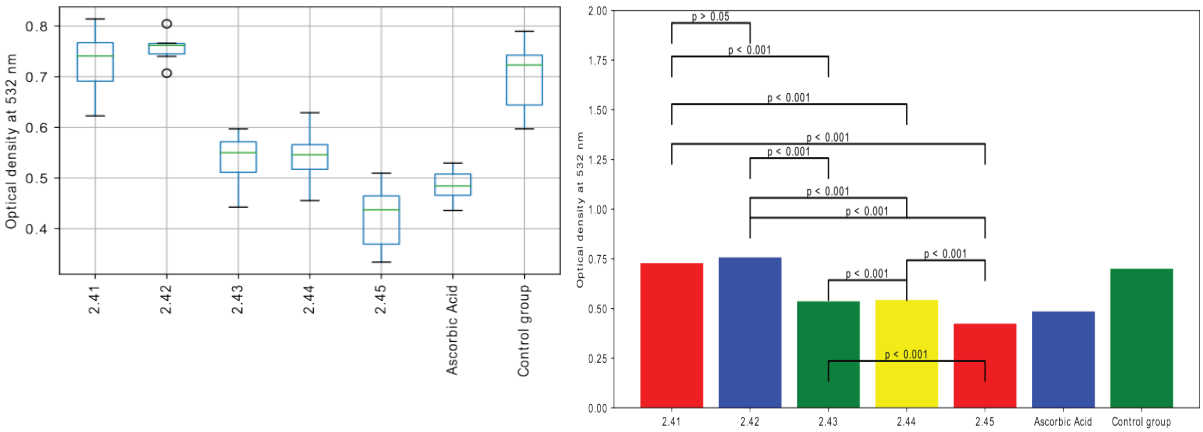

Figure 8. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(heptylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine (**2.41-2.45**)

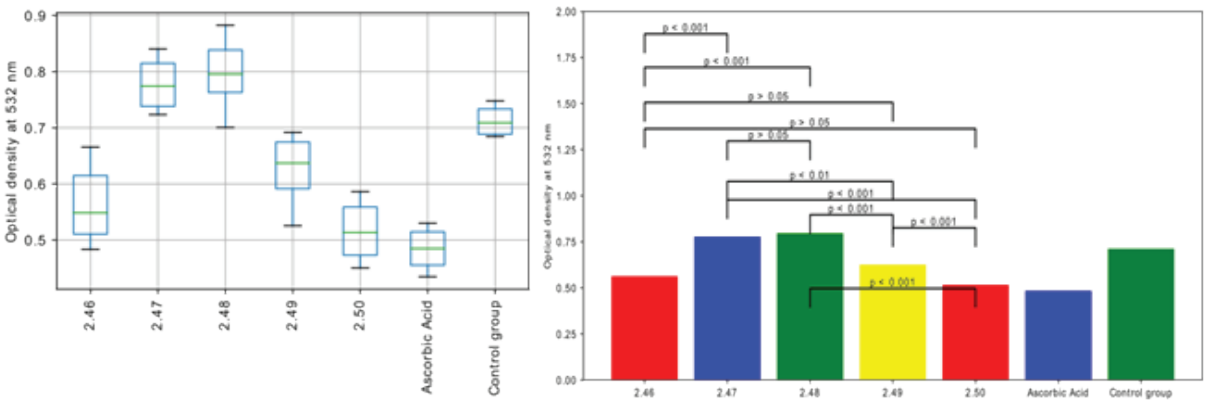

Figure 9. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(octylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine (**2.46-2.50**)

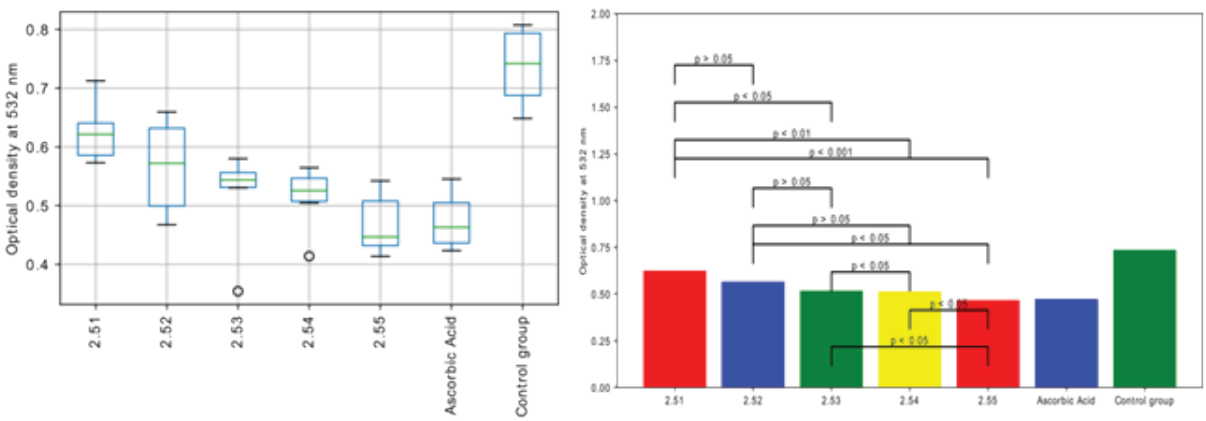

Figure 10. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(nonylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine (**2.51-2.55**)

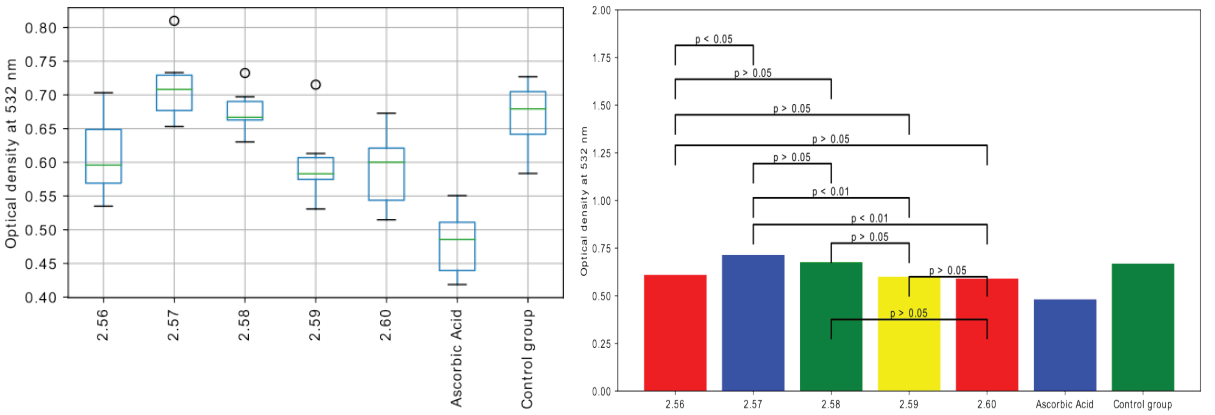

Figure 11. Intergroup and within-group comparisons AOA (Optical density) of 4-((4-R-5-(decylthio)-4*H*-1,2,4-triazol-3-yl) methyl)morpholine (**2.56-2.60**)

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