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Araştırma Makalesi / Research Article

Synthesis, Characterization, of Antioxidant Activity Mannich Bases from 2-Benzoxalinones

Faika BAŞOĞLU[1*], Eda BECER[2], Tuğba ERÇETİN[3], Hayrettin Ozan GÜLCAN[4]

Abstract

This study aimed to synthesize a series of 3-substituted piperazinomethyl derivatives (BzO1-4) via a Mannich reaction and evaluate their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferrous ion- chelation capacity assays. The synthesis process involved precise techniques, and the compounds were characterized using elemental analysis, FT-IR, and ^{1}H-NMR spectroscopy. Notably, BzO-1, featuring a 4-methylphenyl substituent, emerged as a novel compound within this series, demonstrating the highest antioxidant activity among the four derivatives tested. A considerable performance of BzO-1 was evident in its ability to neutralize free radicals and ferric ion-chelating capacity indicating its potential as a potent antioxidant agent. The detailed characterization data provided insights into the molecular structures and electronic environments of the synthesized compounds, which were crucial for understanding their antioxidant mechanisms. These findings suggest that the structural modifications in these piperazinomethyl derivatives significantly influence their antioxidant properties, and the novel BzO-1 compound holds promise for further development and application in antioxidant therapy.

Keywords: Mannich reaction, 1,3-benzoxazole, antioxidant.

2-Benzoksalinonlardan Mannich Bazlarının Sentezi, Karakterizasyonu ve Antioksidan aktivitesi

Öz

Bu çalışma, bir dizi 3-ikameli piperazinometil türevini (BzO1-4) bir Mannich reaksiyonu yoluyla sentezlemeyi ve 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürücü aktivitesi ve demir iyonu şelasyon kapasitesi analizleri kullanarak antioksidan aktivitelerini değerlendirmeyi amaçlamıştır. Sentez süreci hassas teknikler içermiştir ve bileşikler element analizi, FT-IR ve 1H-NMR spektroskopisi kullanılarak karakterize edilmiştir. Özellikle, 4-metilfenil ikamesi içeren BzO-1, bu seride yeni bir bileşik olarak ortaya çıkmış ve test edilen dört türev arasında en yüksek antioksidan aktiviteyi göstermiştir. BzO-1'in serbest radikalleri nötralize etme, demir iyonlarını şelatlama yeteneğinde önemli bir performansın olduğu ortaya çıkmış ve bu da onun güçlü bir antioksidan ajan olarak potansiyelini göstermiştir. Ayrıntılı karakterizasyon verileri, antioksidan mekanizmalarını anlamak için çok önemli olan sentezlenen bileşiklerin moleküler yapıları ve elektronik ortamları hakkında bilgi sağlamıştır. Bu bulgular, piperazinometil türevlerindeki yapısal değişikliklerin antioksidan özelliklerini önemli ölçüde etkilediğini ve yeni BzO-1 bileşiğinin antioksidan tedavide daha fazla geliştirilme ve uygulama için umut vaat ettiğini göstermektedir.

Anahtar Kelimeler: Mannich reaction, 1,3-benzoxazole, antioxidant.

[1]European University of Lefke, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Lefke, North Cyprus, TR-10 Mersin, Türkiye. fabasoglu@eul.edu.tr
[2,3,4]Eastern Mediterranean University, Faculty of Pharmacy, 99628, Famagusta, North Cyprus via Mersin 10, Türkiye. edabecer@yahoo.com tugba.ercetin@emu.edu.tr ozan.gulcan@emu.edu.tr

[*]Sorumlu Yazar/Corresponding Author
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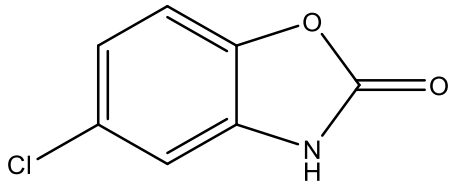
1. Introduction

Benzoxazolone is a heterocyclic organic compound that contains a benzene ring fused with an oxazole ring. It is an important building block in organic chemistry and has diverse applications in the pharmaceutical industry due to its unique structural properties (Zhang *et al.*, 2018). Benzoxazolone derivatives have been used as therapeutic agents for the treatment of various diseases such as inflammation, pain, and neurological disorders (Erdogan *et al.*, 2021). Benzoxazolone derivatives are widely used in drug discovery and development. These compounds have a diverse range of biological activities, including anti-inflammatory, analgesic, antitumor, and antimicrobial activities (Kamal *et al.*, 2020). They also exhibit potent inhibitory effects on certain enzymes, such as cyclooxygenase (COX), which is involved in inflammation and pain (Gökhan-Kelekçi *et al.*, 2009). Moreover, ongoing research into benzoxazolone derivatives aims to explore their potential antioxidant properties, which could address oxidative stress-related conditions and expand their therapeutic applications. Therefore, benzoxazolone is an important heterocyclic compound with diverse pharmaceutical applications. Its derivatives have potent biological activities, and one example of FDA-approved drugs that contain benzoxazolone is Chlorzoxazone. This drug is used as muscle relaxant. Benzoxazolone continues to be an important compound in drug discovery and development.



5-chlorobenzo[*d*]oxazol-2(3*H*)-one

Figure 1. Some FDA approved benzoxazolone derivative, Chlorzoxazone

The damages caused by oxidative stress to biological molecules in the body are effective in a wide range from lipoproteins to membrane lipids, from polyunsaturated fatty acids to nucleotides. Excessive reactive oxygen species (ROS) can disrupt the functionality and structure of cellular compounds, leading to lipid peroxidation and protein oxidation. This process, metabolic oxidative stress, is known to play an important role in the pathogenesis of various diseases and degenerative processes such as inflammation, cancer, dementia and physiological ageing (Çetinkaya *et al.*, 2011). In this context, the importance of antioxidants emerges because these compounds can protect against the harmful effects of ROS and promote cellular homeostasis. The discovery of new synthetic antioxidants may have the potential to offer an effective pharmacological alternative, especially

against oxidative stress, which is expected to play an important role in future therapeutic approaches. Thus, antioxidant research represents a critical area towards the development of novel therapeutic strategies that may provide positive effects on health and disease.

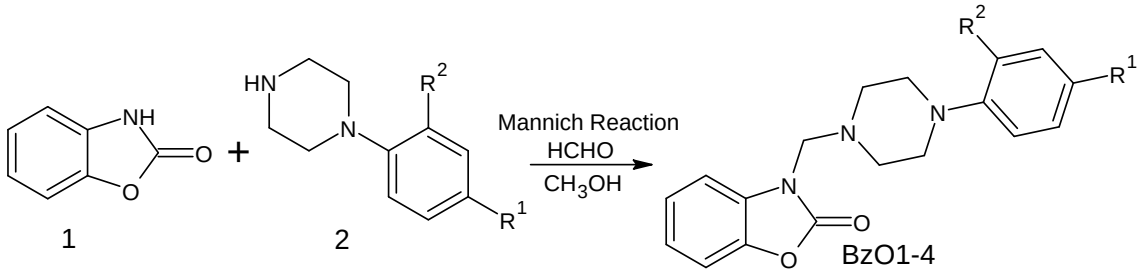
2. Materials and Methods

2.1. Chemistry

2.1.1. Chemicals

All reagents were used as purchased from commercial supplies without further purification. Melting points (°C) were determined by using a Thomas Hoover capillary melting point apparatus (Philadephia, PA. USA) and are uncorrected.

2.1.2. General Synthesis



3-[[4-(2/4-alkylphenyl)piperazin-1-yl]methyl]-1,3-benzoxazol-2(3*H*)-one (BzO1-4)

To 15 ml of methanol solution, a small amount of 0.005 mol of 2-benoxazolinone at 98% purity was added. Following this, 0.005 mol of 1-(2/4-alkylphenyl piperazine) was introduced. Subsequently, a solution of 0.75 ml of 37% formaldehyde in methanol was included. The resulting mixture was left to react for 2 hours at room temperature while being stirred using a stirring machine(Gökhan *et al.,* 2005; Özkanlı, 2004).

2.2. Biological Study – Antioxidant Activity

2.2.1. Ferrous ion-chelating effect

The ferrous ion-chelating effect of the all compounds and reference was estimated by the method of Chua et al. (2008). Briefly, various dilutions of the compounds dissolved in ethanol (80%) were incubated with 2 mM $FeCl_2$ solution (200 µL). The reaction was initiated by the addition of 800 µL of 5 mM ferrozine (Sigma, St. Louis, MO, USA) into the mixture and left standing at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm using a spectrophotometer (Varioskan Flash, Thermo Scientific, USA) against ethanol (80%) as blank. The ratio of inhibition of ferrozine-Fe2+ complex formation was calculated as follows:

$$I\% = \left[\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right] \times 100,$$

where Ablank is the absorbance of the control reaction (containing only $FeCl_2$ and ferrozine), and Asample is the absorbance of the compounds/reference. The reference was ethylenediamine tetraacetic acid (EDTA) were obtained from Sigma Aldrich (USA) in this assay. Analyses were run in triplicates and the results were expressed as average values with S.E.M.

2.2.2. DPPH Radical Scavenging Activity

Blois's UV method was employed to screen the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity. According to this method, 2 mM main stock were prepared in methanol and and the reference molecule (gallic acid) were prepared in methanol and added 20 µL. 180 µL of 0.15 mM DPPH solution in methanol was added to each solution. After 20 min incubation at room temperature, remaining DPPH amount was measured at 520 nm (Varioskan Flash, Thermo Scientific, USA). The percent DPPH radical scavenging activity was calculated through the following formula

I% = [(Acontrol − Asample)/Acontrol] × 100, where Acontrol is the absorbance of the control reaction, and Asample is the absorbance of the extracts/reference. Experiments were run in triplicate and the results s were expressed as average values with S.E.M. (standard error mean).

3. Findings and Discussion

3.1. Chemistry

3-[[4-(4-methylphenyl)piperazin-1-yl]methyl]-1,3-benzoxazol-2(3*H*)-one (BzO-1)

White solid (0.70 g, 43%): Melting point: 160°C. Anal. Calcd. For $C_{19}H_{21}N_3O_2$: C, 74.96; H, 9.15; N, 15.89%. Found: C, 74.93; H, 8.99; N, 15.87%. IR ν (cm^{-1}): 3031 (ar. C-H strecthing), 2948, 2916, 2878, 2817 (al. C-H asymmetrical and symmetrical stretching), 1757 (C=O strecthing), 1484, 1343 (al. C-H asymmetrical and symmetrical), 802 (aromatic 1,4-disubstituted). ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 7.44 (d, J=7.8 Hz, 1H), 7.35 (d, J=8.0 Hz, 1H), 7.23 (t, J=7.7 Hz, 1H), 7.15 (t, J=7.8 Hz, 1H), 6.99 (d, J=8.5 Hz 2H), 6.82 (dd, J=14.4, 8.1 Hz, 2H), 4.74 (s, 2H), 3.08-3.04 (m, 4H), 2.19 (t, J=5.0 Hz, 3H).

3-[[4-(4-nitrophenyl)piperazin-1-yl]methyl]-1,3-benzoxazol-2(3*H*)-one (BzO-2)

Orange solid (0.16 g, 45%): Melting point: >300°C. Anal. Calcd. For $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81%. Found: C, 61.00; H, 5.16; N, 15.79%. IR ν (cm^{-1}): 3073 (ar. C-H strecthing), 2939, 2885, 2839 (al. C-H asymmetrical and symmetrical stretching), 1750 (C=O strecthing), 1596, 1391 (NO2 stretching), 1482, 1330 (al. C-H asymmetrical and symmetrical bending), 825 (aromatic 1,4-disubstituted). ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 7.44 (d, J=7.8 Hz, 1H), 7.35 (d, J=8.0 Hz, 1H), 7.23 (t, J=7.7 Hz, 1H), 7.15 (t, J=7.8 Hz, 1H), 6.99 (d, J=8.5 Hz 2H), 6.82 (dd, J=14.4, 8.1 Hz, 2H), 4.74 (s, 2H), 3.08-3.04 (m, 4H), 2.76 (t, J=5.0 Hz, 4H).

3-[[4-(2-Fluorophenyl)piperazin-1-yl]methyl]-1,3-benzoxazol-2(3*H*)-one (BzO-3)

White solid (1.15 g, 71%): Melting point: 170°C. Anal. Calcd. For $C_{18}H_{18}FN_3O_2$: C, 66.04; H, 5.54; N, 12.84%. Found: C, 66.01; H, 5.57; N, 12.83%. IR ν (cm^{-1}): 3066 (ar. C-H stretching), 2986, 2957, 2881, 2820 (al. C-H asymmetrical and symmetrical stretching), 1757 (C=O stretching), 1484, 1357 (al. C-H asymmetrical and symmetrical), 802 (aromatic 1,4-disubstituted). ^{1}H-NMR (500 MHz) (DMSO-d6/TMS) δ (ppm): 7.44 (d, J=7.8 Hz, 1H), 7.35 (d, J=7.9 Hz, 1H), 7.24 (t, J=7.7 Hz, 1H), 7.15 (t, J=7.8 Hz, 1H), 7.13-7.07 (m, 3H), 7.07-6.89 (m, 2H (1H has arisen from F)), 4.75 (s, 2H), 3.00 (t, J= 4.8 Hz, 4H), 2.80 (t, J=4.8 Hz,4H).

3-[[4-(4-fluorophenyl)piperazin-1-yl]methyl]-1,3-benzoxazol-2(3H)-one (BzO-4)

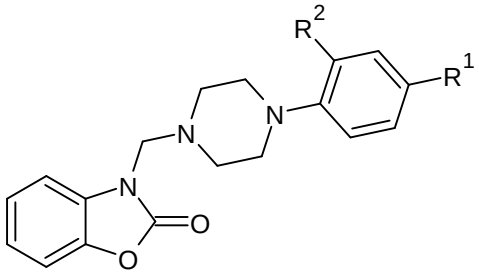
White solid (1.02 g, 63%): Melting point: 172°C. Anal. Calcd. For $C_{18}H_{18}FN_3O_2$: C, 66.04; H, 5.54; N, 12.84%. Found: C, 66.07; H, 5.56; N, 12.85%. IR ν (cm^{-1}): 3150 (ar. C-H stretching), 2939, 2885 (al. C-H asymmetrical and symmetrical stretching), 1755 (C=O stretching), 1484, 1348 (al. C-H asymmetrical and symmetrical), 811 (aromatic 1,4-disubstituted). ^{1}H-NMR (500 MHz)

(DMSO-d_6/TMS) δ (ppm): 7.43 (d, J=7.8 Hz, 1H), 7.34 (d, J=8.0 Hz, 1H), 7.23 (t, J=7.8 Hz, 1H), 7.14 (t, J=7.9 Hz, 1H), 7.01 (t, J=8.6 Hz, 2H), 6.90 (dd, J= 9.1, 4.7 Hz, 2H), 4.74 (s, 2H), 3.05 (t, J= 4.9 Hz, 4H), 2.77 (t, J=4.9 Hz,4H).

Mannich reaction also known as aminoalkylation reaction, at the initial step amine group, is turned into an iminium by nucleophilic addition to formaldehyde and afterward leaving a water molecule. Moreover, Carbonyl compounds can be converted to enol form due to an acidic medium. Thus, the enol form attacks the positively charged carbon of the iminium (Sarpong, 2023).

The melting point and some analysing results of the synthesized 3-[[4-(4-alkylphenyl)piperazin-1-yl]methyl]-1,3-benzoxazol-2(3*H*)-ones using Mannich reaction method are given Table 1.

Table 1. Some physical properties of the synthesized compounds (BzO-1,4).



Compound	R^1	R^2	Formula (M.W.)	Yield (%)	M.p. (C)	Calculated/Found %C	%H	%N
BzO-1	CH_3	H	$C_{19}H_{21}N_3O_2$ (323.39)	43	160	70.57 70.54	6.55 6.62	9.89 9.82
BzO-2	NO_2	H	$C_{18}H_{18}N_4O_4$ (354.36)	45	>300	61.01 61.09	5.12 5.05	15.81 15.76
BzO-3	F	H	$C_{18}H_{18}FN_3O_2$ (327.35)	63	170	66.05 66.01	5.54 5.57	12.84 12.83
BzO-4	H	F	$C_{18}H_{18}FN_3O_2$ (327.35)	71	172	66.05 66.07	5.54 5.56	12.84 12.85

Using FT-IR spectroscopic method functional groups can be determined. Every single atom and atom groups give specific band due to their different vibrations under infrared light. For instance, NH group shows a particular band as a sharp double band around 3200-3500 cm-1 region (Erdik, 2015) whereas tertiary amine group band is disappeared (Erdik, 2015). The synthesized compounds were derived from 2-benzaxozolone scaffold that’s why the final products haven't NH atom group whereas carrying methylene group as a bridge between benzoxazolone and piperazine. Additionally,

C=O group of the lactone give a sharp band around 1790-1750 cm-1 region (Gökhan *et al.,* 2005; Özkanlı, 2004).

Under the light of information given above, the FT-IR spectral data were interpreted. The C=O group band arising by lactone were observed between 1757-1750 cm^{-1} region. Moreover, any NH band wasn't observed as expecting whereas obtaining the aliphatic CH streching and bending bands 2986-2817 cm^{-1} region and 1484-1343 cm^{-1} region, respectively (see suplement materials).

It used a similar approach such as the absence of NH and the presence of aliphatic CH proton peaks to interpret H-NMR spectrums. As the results of previous studies, it was reported that NH group proton peak arising by lactone gives a singlet peak around 12 ppm ("SDBS-Mass," 2023) and moreover, aliphatic CH proton peak is shown between 0-2 ppm. However, some CH proton peaks can be observed around 4-5 ppm because of neighbor atom contribution arising from their electronegativity (Erdik, 2015).

Under the light of information given above, no peak was observed around 12 ppm arising from lactone NH proton whereas obtaining the aliphatic peaks. Methylen proton was observed on 4.75-4.74 ppm as a singlet for all synhtesized compounds (see suplement materials).

3.2. Biological Study – Antioxidant Activity

Among the synthesized derivatives, 4-methylphenyl substituted (BzO-1) was original and exhibited the best antioxidant activity with an IC_{50} value of 8.16 ± 0.007 µM via metal-chelation mechanism. Previous studies have demonstrated the analgesic effects of 2-fluorophenyl derivatives, which are commercially used analgesics (Zygmunt et al, 2015). The study aimed to explore the antioxidant properties of these compounds and found a significant correlation between antioxidant and analgesic activities, particularly in BzO-1, which displayed notable ion chelating activity. This highlights the potential dual therapeutic benefits of these compounds, combining antioxidant and analgesic properties, which can be advantageous in managing conditions involving oxidative stress and pain.

Table 2. DPPH free radical-scavenging activity (200 µM) and ferric ion chelating effect (inhibition % ± S.E.M) of synthesized compounds at 100 µM & 200 µM reaction concentration (inhibition % ± S.E.M)

Compound code	DPPH (200 µM)	ION CHELATING (100 µM)	ION CHELATING (200 µM)
BzO-1	8.16 ± 0.007	53.05± 0.011	**56.63± 0.041**
BzO-2	5.03 ± 0.002	19.02 ± 0.009	26.21 ± 0.012
BzO-3	4.95 ± 0.011	20.25 ± 0.029	27.37 ± 0.079
BzO-4	6.24 ± 0.003	37.02± 0.062	**54.45± 0.028**
EDTA	-	35.01 ± 0.014	68.65 ± 0.011
GALLIC ACID	60.95 ± 0.001	-	-

4. Conclusions and Recommendations

In conclusion, the synthesis of a series of 3-substituted piperazinomethyl derivatives (BzO1-4) via the Mannich reaction was successfully accomplished, and their antioxidant activities were thoroughly investigated. The synthesized compounds were characterized by elemental analysis, FT-IR, and ^{1}H-NMR spectroscopy, confirming their structures. Among the derivatives, BzO-1, featuring a 4-methylphenyl substituent, emerged as a novel compound with the highest antioxidant activity. The results from DPPH radical scavenging and ferric ion-chelating capacity assays demonstrated that BzO-1 exhibits a considerable ability to neutralize free radicals and chelate metal ions. These findings suggest that structural modifications in these piperazinomethyl derivatives significantly influence their antioxidant properties.

Future research should focus on further exploring the structure-activity relationship (SAR) of these derivatives to enhance their antioxidant efficacy. Additionally, in vivo studies are recommended to evaluate the bioavailability and therapeutic potential of BzO-1. Investigating other biological activities, such as anti-inflammatory and anticancer properties, could provide a comprehensive understanding of these compounds' pharmacological profiles. Finally, the development of formulation strategies to improve the stability and delivery of BzO-1 in biological systems would be beneficial for potential therapeutic applications.

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Authors' Contributions

Contribution of the authors to the article should be indicated. (For example: All authors contributed equally to the study.)

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

References

Berg, J. M., Tymoczko, J. L., Gatto, G. J., & Strauss, C. J. (2019). *Biochemistry* (8th ed.). W.H. Freeman and Company.

Blois, M.S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical.*Nature*, 181, 1199–1200.

Cetinkaya, Y., Göçer, H., Menzek, A., & Gülçin, İ. (2012). Synthesis and antioxidant properties of (3, 4-dihydroxyphenyl)(2, 3, 4-trihydroxyphenyl) methanone and its derivatives. *Archiv der Pharmazie*, 345(4), 323-334.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315, 161–169.

Ercetin, T., Senol, F.S., Orhan, I.E. and Toker, G. (2012). Comparative assessment of antioxidant and cholinesterase inhibitory properties of the marigold extracts from Calendula arvensis L. and Calendula officinalis L. *Industrial Crops and Products*, 36(1), 203-208.

Erdik, E. (2015). *Organik Kimyada Spektroskopik Yöntemler*. Gazi Kitabevi, Ankara.

Erdogan, M., Kilic, B., Sagkan, R. I., Aksakal, F., Ercetin, T., Gulcan, H. O., & Dogruer, D. S. (2021). Design, synthesis and biological evaluation of new benzoxazolone/benzothiazolone derivatives as multi-target agents against Alzheimer's disease. *European Journal of Medicinal Chemistry*, *212*, 113124.

FDA. (2019). Eperisone: Drug information. Retrieved from https://www.fda.gov/drugs

FDA. (2020). Piroxicam: Drug information. Retrieved from https://www.fda.gov/drugs

Gibaldi, M. (2016). *Drug delivery: Principles and applications* (2nd ed.). CRC Press.

Gökhan, N., Köksal, M., Küpelİ, E., Yeşilada, E., & Erdoğan, H. (2005). Some New Mannich Bases of 5-Methyl-2-Benzoxazolinones With Analgesis and Anti-Inflammatory Activities. *Turkish Journal of Chemistry*. Retrieved from https://journals.tubitak.gov.tr/chem/vol29/iss4/13.

Gökhan-Kelekçi, N., Köksal, M., Ünüvar, S., Aktay, G., & Erdoğan, H. (2009). Synthesis and characterization of some new 2 (3H)-benzoxazolones with analgesic and antiinflammatory activities. *Journal of enzyme inhibition and medicinal chemistry*, *24*(1), 29-37.

Kamal, U. D. D. I. N., Javed, N. M., & Arun, K. U. M. A. R. (2020). Biological potential of benzoxazole derivatives: an updated review. *Asian J. Pharm. Clin. Res*, *13*(8), 28-41.

Koksal, M., Gokhan, N., Kupeli, E., Yesilada, E., & Erdogan, H. (2007). Analgesic and antiinflammatory activities of some new Mannich bases of 5-nitro-2-benzoxazolinones. *Arch Pharm Res*, *30*(4), 419-424. doi:10.1007/BF02980214.

Moffat, A. C., Osselton, M. D., Widdop, B., & Watts, J. (2011). *Clarke's analysis of drugs and poisons* (Vol. 3, p. 533). London: Pharmaceutical press.

O'Neil, M. J. (Ed.). (2013). *The Merck index: an encyclopedia of chemicals, drugs, and biologicals*. RSC Publishing.

Özkanlı, F. (2004). Synthesis Of Some New Mannich Bases Of 6-Acyl-5-Chloro-2-Benzoxazolinones. *Research Article*, 80-90.

Pobudkowska A, Domańska U. Study of pH-dependent drugs solubility in water. Chemical Industry and Chemical Engineering Quarterly. 2014;20(1):115-26

Sarpong, R. P., F. Reeves, J.. (2023). Mannich Reaction. *Photochemistry*. Retrieved from https://www.organic-chemistry.org/namedreactions/mannich-reaction.shtm

SDBS-Mass. (2023). Retrieved from https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi

art 2017).