RESEARCH ARTICLE

Pharmacological Screening of Some Novel 3,5-Disubstituted Isoxazoles Derivatives of Dibromochalcones

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ABSTRACT

Background: The preparation of some novel 3, 5-disubstituted isoxazole derivatives of dibromochalcones is encouraged by the study. When hydroxylamine hydrochloride and α-β chalcone dibromide combine, a new technique for synthesizing 3,5-disubstituted isoxazoles is produced. Bromine or N-BromoSuccinimide can be used to produce compounds with low polarity like carbon tetrachloride, chloroform and dichloromethane; however the product obtained are very little. Tetrabutylammonium tribromide (TBABr3), thus, induces chalcones to bromate regioselectively and produces a high yield without polymerisation. TBABr3 is not toxic or corrosive, unlike bromine, and it is simple to utilise in mild circumstances. Tetrabutyl ammonium bromide and sodium bromide are dissolved in an aqueous solution at room temperature, then hydrobromic acid is added to create TBABr3.Motivated by the aforementioned information, we intended to create further isoxazole derivatives and assess their ability to reduce inflammation. Focused on a range of 3,5-disubstituted isoxazole derivatives of α-β dibromochalcones, the current study was created by reacting α-β dibromochalcones with hydroxylamine hydrochloride.

Methods: Tetrabutyl Ammonium Tribromide (TBABr3) was used to react with the corresponding chalcones to produce a high yield of α-β dibromochalcones that are regioselectively brominated without polymerisation. After this characterization, antioxidant and pharmacological qualities were assessed.

Results: Many 3,5-disubstituted isoxazoles were created by reacting hydroxylamine hydrochloride with chalcone dibromides. The substances were all characterized using elemental analysis, NMR, and IR. The product's infrared spectra revealed that the carbonyl group's peak at 1680 cm–1 was absent. A strong singlet at δ = 8.1 ppm was seen in the 1H NMR spectra. This single proton corresponded to the C (3)-H of the isoxazole ring. The ability of antioxidants to scavenge free radicals was assessed using DPPH.

Conclusion: Comparing the compounds V5I5 and V7I7 to the standard, they showed modest antioxidant activity. When the synthetic compounds were evaluated for their pharmacological activities, substances V2I2 and V4I4 outperformed the reference.

Keywords: Isoxazoles, Dibromochalcones, Substituted Isoxazoles, Tetrabutylammoniumtribromide ($TBABr_3$), Anti-inflammatory and Antioxidant activity

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INTRODUCTION

An important part of the body's immunological defences is inflammation. It may be persistent or acute. Pain, heat, oedema, and other symptoms are possible. Depending on the underlying cause, treatments may vary. When the body encounters a foreign object, such as a disease, irritation, or thorn, it may deploy cells to defend itself. Infection-causing bacteria, viruses, and other organisms are known as pathogens. The body is capable of misinterpreting its own cells or tissues as harmful. This response may result in autoimmune diseases. NAIDS, or non-steroidal anti-inflammatory medications, are frequently used in conjunction with other treatments for a range of illnesses. They do this by inhibiting the pathway that leads to the synthesis of prostaglandins, which controls inflammation and pathological diseases. Two recognized cyclooxygenases (COX-1 and COX-2) catalyse this route.[1-3] The majority of inflammatory symptoms, including pain, redness, tissue infiltration, and oedema, are caused by COX-2, whereas COX-1 is in charge of maintaining the integrity of the stomach and kidney cells.[4] Non-selective anti-inflammatory drugs sporadically block both COXs, which may affect stomach and renal function. Thus, the need for selective COX-2 inhibitors is very high. The Coxibs family, which includes celecoxib[5], etricoxib, validocoxib, and rofecoxib, is regarded as a remarkable accomplishment in this field because of their excellent selectivity towards COX-2. However, validocoxib and rofecoxib continue to have cardiac side effects, which is why they were taken off the market.[6-7] Because of a side pocket that has the capacity to hold a big aryl, COX-2 vol is greater than COX-1 vol. Therefore, the volume of prepared compounds would rise with the use of preferable isoxazoles, aryl substituted furoxan, or fused isoxazoles. To overcome these cardiac issues, certain aryls, such as furoxan[8-10], are well-known for their metabolic

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breakdown, which can result in the physiological production of nitroxyl (HNO), a type of nitric oxide (NO).[11] Additionally, isoxazole, a preferred anti-inflammatory pharmacophore, may be able to preserve COX-2 selectivity with little adverse effects.[12] Heterocyclic compounds have attracted a lot of attention because of their capacity to close the gap between the chemical and biological sciences. Currently, these compounds are the focus of a great deal of contemporary research worldwide. The chemistry of isoxazoles has been an intriguing area of research for decades due to its well-known effects as an analgesic[13], anti-inflammatory[14], anticancer[15,16], antibacterial[17], antiviral, anticonvulsant, depressed, and immunosuppressive.[18–20] The literature review revealed that when different groups are substituted on the isoxazole ring, different actions are imparted. Isoxazoles are a significant class of heterocyclic compounds with unique features that have developed over time. Derivatives of isoxazoles have proven to be a valuable component in the synthesis of several chemical molecules. It is possible to transform isoxazoles into a number of significant moieties, including -hydroxy ketones, -amino alcohols,-unsaturated oxime, and -hydroxy nitriles. In addition, a wide range of established pharmacological and biological characteristics, such as analgesic, hypoglycemic, anti-inflammatory, antibacterial, and anticancer effects, are possessed by isoxazole derivatives.[21–25] Derivatives of 3,5-diarylisoxazole are also powerful therapeutic agents with anti-inflammatory and analgesic properties, as well as the ability to inhibit cyclooxygenase-II and treat tumours. These substances are also helpful in treating psoriasis, rheumatoid arthritis, and malignancies that are linked to angiogenesis.[26–31] Considering the aforementioned, the current investigation promotes the creation of 3,5-disubstituted isoxazole derivatives using α-β chalcone dibromides. When hydroxylamine hydrochloride and dibromochalcones combine, a new technique for synthesizing 3,5-disubstituted isoxazoles is produced. Bromine or N-BromoSuccinimide can be used to produce compounds with low polarity like carbon tetrachloride, chloroform and dichloromethane; however the product obtained are very little.[32] Tetrabutylammonium tribromide (TBABr3), thus, induces chalcones to bromate regioselectively and produces a high yield without polymerisation. TBABr3 is not toxic or corrosive, unlike bromine, and it is simple to utilize in mild circumstances. Tetrabutyl ammonium bromide and sodium bromide are dissolved in an aqueous solution at room temperature, and then hydrobromic acid is added to create TBABr3. Motivated by the aforementioned information, we intended to create further isoxazole derivatives and assess their ability to reduce inflammation.

MATERIALS AND METHODS

Synthesis

The uncorrected melting points of the synthesized compounds were determined. To ensure purity and track the reaction, TLC was used and IR and 1HNMR spectra was also recorded. As an internal standard, tetramethylsilane (TMS) is used to record the chemical changes in parts per million (δ). Hertz (Hz) is used to express coupling constants (J). We bought all of the chemicals and reagents from Sigma Aldrich and Merck.

Preparation of various 3,5 Disubstituted Isoxazoles from Dibromochalcones

For around four hours, a mixture of sodium acetate (0.001 mol), hydroxylamine hydrochloride (0.001 mol), and chalcone dibromide (0.0005 mol) in 35 millilitres of ethanol were refluxed. After that, the mixture was extracted using dichloromethane and the resultant organic extract was filtered and dried over anhydrous sodium sulphate. Pure isoxazole was prepared by evaporating dichloromethane in a vacuum and then purified by performing column chromatography using ether–ethylacetate as the eluent.

5-(2-methoxynaphthalen-6-yl)-3-phenylisoxazole (V1I1)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.31 (m, 4H, ArH); 8.29 (s, 1H, Isoxazole-H-3); 6.97-7.66 (m, 6H, ArH); 3.73 (s, 1H, $–OCH_3$); Anal. Calcd for $C_{20}H_{15}NO_2$; C 79.72, H 5.02 Found C 79.70, H 5.00

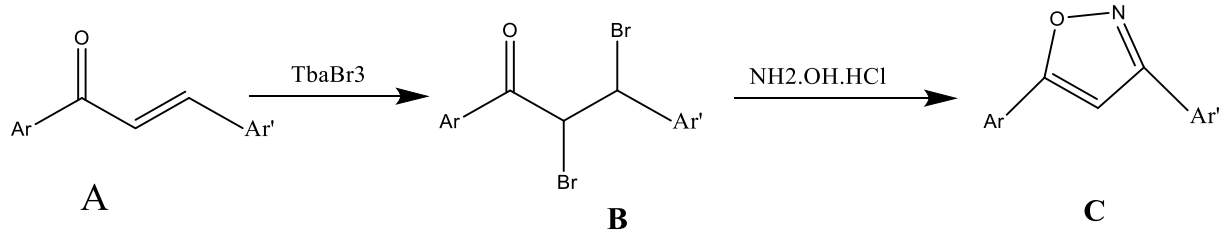
3-(4-chlorophenyl)-5-(2-methoxynaphthalen-6-yl)isoxazole (V2I2)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.33 (d, 2H, ArH, J = 7.6 Hz); 7.42 (d, 2H, ArH, J = 7.1 Hz); 8.28 (s, 1H, Isoxazole-H-3); 6.97-7.86 (m,6H,ArH); 3.72 (s , 1H, $–OCH_3$) ; Anal. Calcd for $C_{20}H_{14}ClNO_2$; C 71.54, H 4.20 Found C 71.50, H 4.19

5-(2-methoxynaphthalene-6-yl)-3-(4-nitrophenyl)isoxazole (V3I3)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.74 (d, 2H, ArH, J = 7.2 Hz); 8.25 (d, 2H, ArH, J = 7.2 Hz); 8.26 (s, 1H, Isoxazole-H-3); 6.96-7.85 (m,6H,ArH); 3.71 (s , 1H, $–OCH_3$) ; Anal. Calcd for $C_{20}H_{14}N_2O_4$; C 69.36, H 4.07 Found C 69.28, H 3.99

5-(2-methoxynaphthalen-7-yl)-3-(4-



Scheme 1: Scheme for synthesis of 3,5-disubstituted isoxazoles

Table 1: Results of 3,5 disubstituted Isoxazoles as Antioxidant.

Sr. No.	Product	Percent Inhibition for concentration (µg/ml)					IC_{50} value
		10	20	40	60	100	
1	V1I1	1.34	2.98	12.72	18.29	22.61	200.26
2	V2I2	1.20	7.12	13.95	16.83	21.92	221.04
3	V3I3	1.93	3.10	16.23	19.51	22.79	198.26
4	V4I4	23.1	33.39	38.54	42.68	52.56	86.92
5	V5I5	25.8	38.11	48.97	53.33	69.81	52.31
6	V6I6	7.95	12.03	21.61	32.19	40.03	120.33
7	V7I7	22.44	34.38	46.03	54.71	68.10	56.17
8	Ascorbic acid (Standard)	51.14	64.04	69.38	84.12	96.03	2.12

methoxyphenyl)isoxazole (V4I4)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.37 (d, 2H, ArH, J = 7.1 Hz); 6.83 (d, 2H, ArH, J = 7.1 Hz); 8.27 (s, 1H, Isoxazole-H-3); 6.94-7.83 (m,6H,ArH); 3.72 (s , 1H, $–OCH_3$) Elemental analysis: Calculated for $C_{21}H_{17}NO_3$; C 76.12, H 5.17 Found C 76.10, H 5.14

3-(4-fluorophenyl)-5-(2-methoxynaphthalen-7-yl)isoxazole (V5I5)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.46 (d, 2H, ArH, J = 7.2 Hz); 7.03 (d, 2H, ArH, J = 7.2 Hz); 8.25 (s, 1H, Isoxazole-H-3); 6.97-7.86 (m,6H,ArH); 3.72 (s , 1H, $–OCH_3$) ;Anal. Calcd for $C_{20}H_{14}FNO_2$; C 75.22, H 4.42 Found C 75.19, H 4.38

5-(2-methoxynaphthalen-7-yl)-3-p-tolylisoxazole (V6I6)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.36 (d, 2H, ArH, J = 7.1 Hz); 7.12 (d, 2H, ArH, J = 7.2 Hz); 8.26 (s, 1H, Isoxazole-H-3); 2.35 (s , 1H, $–CH_3$); 6.95-7.84 (m,6H,ArH); 3.72 (s , 1H, $–OCH_3$) ;Anal. Calcd for $C_{21}H_{17}NO_2$; C 79.98, H 5.43 Found C 79.89, H 5.37

4-(5-(2-methoxynaphthalen-7-yl)isoxazol-3-yl)phenol (V7I7)

IR (KBr, cm^{-1}): Absence of peak in CO region; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.31 (d, 2H, ArH, J = 7.1 Hz); 6.79 (d, 2H, ArH, J = 7.2 Hz); 8.24 (s, 1H, Isoxazole-H-3); 4.98 (s ,1H, $–CH_3$); 6.94-7.82 (m,6H,ArH); 3.71 (s , 1H, $–OCH_3$) ;Anal. Calcd for $C_{20}H_{15}NO_3$; C 75.70, H 4.76 Found C 75.64, H 4.72

RESULTS AND DISCUSSION

Chemistry

By reaction of various chalcones [A] with tetrabutylammonium tribromide (TBABr3), several derivatives of dibromochalcones were produced. Different 3,5-disubstituted isoxazoles [C] were produced by treating these chalcone dibromides [B] with hydroxylamine hydrochloride (Scheme 1). Every substrate reacted identically and produced a good yield between 64 and 78%. The compounds underwent vacuum drying and recrystallization from ethanol to achieve purification. Elements analysis, 1NMR, and infrared spectroscopy were used to describe the synthesized compounds.

Biological Evaluation

Evaluation of Antioxidant activity

Through the use of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging technique, the antioxidant activity of the compounds was discovered. 95% methanol was combined with the provided chemical to create the final stock solution (100µg/ml). Various concentration solutions (10µg/ml to 100µg/ml) were made using this stock solution. Ascorbic acid served as the standard, and different ascorbic acid concentrations were made to correspond to the test compound. 2.5 milliliters of sample solution at different concentrations were combined with 1ml of 0.3 millimol DPPH methanol solution to create the final mixture, which was then left to react at room temperature. After an incubation period of 15 minutes at 37^0C, the absorbance at 517nm was determined. Without the test chemical, the control reading was also noted. The capacity of the synthesized compounds to quench DPPH, using ascorbic acid as a standard, was used to assess their free radical scavenging activity. Table 1 presents the

Table 2: Results of 3,5-disubstituted Isoxazole derivatives as Anti-inflammatory.

Product	Paw volume (mm) after time (hrs)*#					%
	0	1	2	3	4	Inhibition
V1I1	0.72±0.044	1.12±0.0728	1.31±0.067	1.42±0.052	1.49±0.023	46.97
V2I2	0.63±0.022	1.18±0.036	1.27±0.071	1.35±0.024	1.41±0.020	49.82
V3I3	0.86±0.014	1.28±0.033	1.39±0.029	1.42±0.027	1.76±0.071	37.36
V4I4	0.43±0.031	0.63±0.026	0.79±0.073	0.92±0.030	1.02±0.032	63.70
V5I5	0.81±0.039	1.19±0.099	1.24±0.067	1.38±0.043	1.48±0.081	47.33
V6I6	0.97±0.034	1.24±0.057	1.37±0.083	1.47±0.098	1.56±0.057	44.48
V7I7	0.73±0.051	1.26±0.076	1.38±0.092	1.48±0.081	1.62±0.052	42.34
Control	0.71±0.034	0.95±0.096	1.82±0.046	2.02±0.034	2.81±0.023	….
Diclofenac Sodium	0.56±0.032	0.66±0.012	0.83±0.032	0.82±0.02	0.78±0.001	72.24

*expressed as mean ± SEM (standard error mean)

#calculated by using one way ANOVA followed by Dunnet's test

findings. Every synthetic molecule exhibited lower potency compared to the reference. Comparing the compounds V5I5 and V7I7 to the standard, they showed modest antioxidant activity.

***In vivo* anti-inflammatory activity**

The paw oedema method caused by carrageenan was utilized to assess the compounds' anti-inflammatory properties. The anti-inflammatory effect was assessed in adult male rats weighing approximately 250g. Ten groupings were used to classify the animals. Every group has six creatures in it. The various doses were administered to each group prior to treatment. Each rat's left hind paw's sub-plantar region was given 0.1 ml of a 1% carrageenan suspension after an hour, and the paw volume was measured at 0, 1, 2, 3, and 4 hours later using a Plethysmometer. For every time interval, the mean ± SEM of the treated and control animals was computed, compared, and statistically examined. Diclofenac sodium was administered as standard. The adult male rats were given free access to water during their overnight fast. The normal medication concentration was 20 mg/kg. The synthesised compounds were taken orally at a dose of 150 mg/kg. A carrageenan 1% saline suspension was made. To create oedema, 0.05 ml of this suspension was injected into the rat's left hindpaw's planter tissue. An identical volume of saline was injected into the animals as a control. Rats' paw volume was measured using a Plethysmograph. The results are displayed in Table 2. The anti-inflammatory properties of the substances **V2I2** and **V4I4** are quite impressive.

CONCLUSION

Many 3,5-disubstituted isoxazoles were created by reacting hydroxylamine hydrochloride with chalcone dibromides. The substances were all characterised using elemental analysis, NMR, and IR. The product's infrared spectra revealed that the carbonyl group's peak at 1680 cm–1 was absent. A strong singlet at δ = 8.2 ppm was seen in the 1H NMR spectra. This single proton corresponded to the C(3)-H of the isoxazole ring. The ability of antioxidants to scavenge free radicals was assessed using DPPH. In comparison to the reference, the compounds V5B5I5 and V7B7I7 showed modest antioxidant activity. When synthetic isoxazoles were evaluated for their ability to reduce inflammation in vivo, substances V2I2 and V4I4 performed well when compared to the reference.

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REFERENCES

1. Alsalameh, S, Burian, M, Mahr G, Woodcock BG, Geisslinger G. The pharmacological properties and clinical use of valdecoxib, a new cyclo-oxygenase-2-selective inhibitor. Aliment. Pharmacol. Ther. 2003; 17: 489–501. DOI: 10.1046/j.1365-2036.2003.01460.x.
2. Fox RI, Herrmann ML, Frangou CG, Wahl GM, Morris RE, Strand V, Kirschbaum BJ. Mechanism of action for leflunomide in rheumatoid arthritis. Clin. Immunol. 1999; 93:198–208. DOI: 10.1006/clim.1999.4777
3. Christian B. M. Poulie, Na Liu, Anders A. Jensen, Lennart Bunch. Design, Synthesis, and Pharmacological Characterization of Heterobivalent Ligands for the Putative 5-HT2A/mGlu2 Receptor Complex. Journal of Medicinal Chemistry. 2020;63 (17):9928-9949. DOI.org/10.1021/acs.jmedchem.0c01058
4. Ryng S, Głowiak T. Nucleophilic Substitution of an Acyl Azide: General Method for the Preparation of 5-Amino-3-methyl-4-isoxazolecarboxylic Acid Amides and Hydrazides. Synth. Commun. 1997, 27, 1359–1368. DOI.org/10.1080/00397919708006065
5. Ryng S, Machoń Z, Wieczorek Z, Zimecki M. Synthesis and immunological activity of new 5-amino-3-methyl 4-amido and 4-ureilene isoxazole derivatives. Pharmazie. 1999;54:359–361. DOI:10.1002/chin.199940110
6. Ryng S, Zimecki M, Fedorowicz A, Jezierska A. Reactions of 5-amino-3-methylisoxazole-4-carboxylic acid hydrazide with carbonyl compounds: Immunological activity and QSAR studies of products. Arch. Pharm. 2001; 334: 71–78. DOI.org/10.1002/1521-4184(200103)334:3<71::AID-ARDP71>3.0.CO;2-I
7. Jezierska A, Mączyński M, Koll A, Ryng S. Structure activity investigations of 5-substituted 3-methylisoxazole[5, 4-d]1, 2, 3-triazin-4-one derivatives. Arch. Pharm. 2004; 337: 81–89. DOI: 10.1002/ardp.200300757
8. Ryng S, Sonnenberg Z, Zimecki M. RM-11, a new izoxasole derivative, is a potent stimulator of the humoral and cellular immune responses in mice. Arch. Immunol. Ther. Exp. 2000; 48: 127–131. DOI: 10.3390/molecules23102724
9. Ryng S, Machon Z, Wieczorek Z, Zimecki M, Głowiak T. Synthesis and Structure Elucidation of 5-Aminomethinimino-3-methyl-4-isoxazolecarboxylic Acid Phenylamides and Their Immunological Activity. Arch. Pharm. 1997; 330: 319–326. DOI: 10.1002/ardp.19973301102
10. Zimecki M, Artym J, Ryng S, Obmińska-Mrukowicz B. RM-11, an isoxazole derivative, accelerates restoration of the immune function in mice treated with cyclophosphamide. Pharmacol. Rep. 2008; 60: 183–189. DOI:18443379
11. Zimecki M, Artym J, Kocięba M, Obmińska-Mrukowicz B, Mączyński M, Ryng S. Restoration of immune system function is accelerated in immunocompromised mice by the B-cell-tropic isoxazole R-11. Pharmacol. Rep. 2012; 64: 403–411. DOI:10.1016/s1734-1140(12)70781-8
12. Zimecki M, Artym J, Kocięba M, Obmińska-Mrukowicz B, Mączyński M, Ryng S. Immune function in cyclophosphamide-treated mice is restored by the T-cell-tropic isoxazole derivative R-13. J.

Immunotoxicol. 2015; 12: 322–329. DOI: 10.3109/1547691x.2014.978486
13. Ryng S, Zimecki M, Mączyński M, Chodaczek G, Kocięba M. Immunosuppressive activity of an isoxazolo[5,4-e]triazepine-compound RM33 I. Effects on the humoral and cellular immune response in mice. Pharmacol. Rep. 2005; 57: 195–202. DOI:10.1016/s1734-1140(12)70787-6
14. Ryng S, Zimecki M, Mączyński M, Chodaczek G, Kocięba M. Immunosuppressory activity of an isoxazolo[5,4-e]triazepine-compound RM-33 II. Effects on the carrageenan-induced inflammation. Pharmacol. Rep. 2006; 58: 236–241. DOI:10.1016/s1734-1140(12)99789-6
15. Ryng S, Zimecki M, Mączyński M, Chodaczek G, Kocięba M. Closely related isoxazoles may exhibit opposite immunological activities. Acta Pol. Pharm. 2008; 65: 793–794. DOI: 10.3109/1547691x.2014.878484
16. Ryng S, Zimecki M, Mączyński M, Chodaczek G, Kocięba M. Synthesis, immunomodulating effects and structure-activity relationships of new N-phenyl-5-amino-3-methylisoxazole-4-carboxamides. Eur. J. Med. Chem. 1998; 33: 831–836. DOI: 10.1002/(sici)1521-4184(19995)332:5<158::aid-ardp158>3.0.co;2-n
17. Ryng S, Zimecki M, Mączyński M, Chodaczek G, Kocięba M. Immunomodulating action and structure-activity relationships of substituted phenylamides of 5-amino-3-methylisoxazole-4-carboxylic acid. Arch. Pharm. 1999; 332: 158–162. DOI: 10.1002/(sici)1521-4184(19995)332:5<158::aid-ardp158>3.0.co;2-n
18. Ryng S, Zimecki M, Mączyński M, Chodaczek G, Kocięba M. Koll, A. Immunological activity of new heterocyclic amides of 5-amino-3-methylisoxazole-4-carboxylic acid. Pol. J. Pharmacol. 1999; 51: 257–262. DOI: 10.3109/1547691x.2014.878484
19. Mączyński M, Artym J, Kocięba M, Sochacka-Ćwikła A, Drozd-Szczygieł E, Ryng S, Zimecki M. Synthesis and immunoregulatory properties of selected 5-amino-3-methyl-4-isoxazolecarboxylic acid benzylamides. Acta Pol. Pharm. 2016; 73: 1201–1211. DOI: 10.3390/molecules23102724
20. Mączyński M, Zimecki M, Taraszkiewicz M, Ryng, S. Synthesis, immunological activity and computational study of 5-amino-3-methyl-4-isoxazolecarboxylic acid semicarbazides and thiosemicarbazides. Acta Pol. Pharm. 2008; 65: 543–549. DOI: 10.3390/molecules23102723
21. Mączyński M, Zimecki M, Taraszkiewicz M, Ryng, S. Structure and immunological activity of disubstituted thiosemicarbazide isoxazole derivative. Acta Pol. Pharm. 2004; 61: 82–83. DOI: 10.3390/molecules23102724
22. Mączyński M, Zimecki M, Taraszkiewicz M, Ryng, S. A new class of isoxazole derivatives: The M 1-9 series of compounds with immunotropic activity. Acta Pol. Pharm. 2008; 65: 241–244. DOI: 10.3109/1547691x.2014.878484
23. Mączyński M, Zimecki M, Taraszkiewicz M, Ryng, S. Jodkowski JT. New lead structures in the isoxazole system: Relationship between quantum chemical parameters and immunological activity. Acta Pol. Pharm. 2014; 71: 71–83. DOI: 10.3209/1547691x.2014.878476
24. Shaw G, Sugowdz G. ISO Oxazolones. Part VI. The hydrogenation of 5-aminoisooxazoles. A new synthesis of pyrimidines. J. Chem. Soc. 1954; 23: 665–668. DOI: 10.3390/molecules23071545
25. Baba H, Hori I, Hayashi T, Midorikawa H. Reactions of α-Cyano-β-methoxy-β-alkylacrylic Esters with Hydrazine and Hydroxylamine. Bull. Chem. Soc. Jpn. 1969; 42: 1653–1659. DOI:10.1002/chin.200547164
26. Regiec A, Płoszaj P, Ryng S, Wojciechowski P. Vibrational spectroscopy of 5-amino-3-methyl-4-isoxazolecarbohydrazide and its N-deuterated isotopologue. Vib. Spectrosc. 2014; 70: 125–136. DOI:10.1016/j.vibspec.2013.11.012
27. Regiec A, Płoszaj P, Ryng S, Wojciechowski P. Infrared spectra and other properties predictions of 5-amino-3-methyl-4-isoxazolecarbohydrazide with electric field simulation using CPC model. J. Mol. Struct. 2018, 1161, 320–338. DOI: 10.3390/molecules23071545
28. Fragoso, YD, Brooks JBB. Leflunomide and teriflunomide: Altering the metabolism of pyrimidines for the treatment of autoimmune diseases. Expert Rev. Clin. Pharmacol. 2015; 8: 315–320. DOI: 10.1586/17512433.2015.1019343
29. Patrick GL. An Introduction to Medicinal Chemistry; OUP Oxford: Oxford, UK, 2013; ISBN 978-0-19-969739-7.
30. Laufer SA, Margutti S, Fritz MD. Substituted isoxazoles as potent inhibitors of p38 MAP kinase. Chem. Med. Chem. 2006; 1: 197–207. DOI: 10.1002/cmdc.200500025
31. Khan KM, Mughal UR, Khan MTH, Perveen S. Oxazolones: New tyrosinase inhibitors; synthesis and their structure–activity relationships. Bioorg. Med. Chem. 2006; 14: 6027–6033. DOI: 10.1016/j.bmc.2006.05.014
32. Mączyński M, Artym J, Kocięba M, Kochanowska I, Ryng S, Zimecki M. Anti-inflammatory properties of an isoxazole derivative—MZO-2. Pharmacol. Rep. 2016; 68: 894–902. DOI: 10.1016/j.pharep.2016.04.017
33. Mukerjee N, McGinnis KM, Gnegy ME, Wang KK. Caspase-mediated calcineurin activation contributes to IL-2 release during T cell activation. Biochem. Biophys. Res. Commun. 2001; 285: 1192–1199. DOI: 10.1006/bbrc.2001.5278
34. Drynda A, Obmińska-Mrukowicz B, Zaczyńska E, Zimecki M, Kochanowska I, Ryn S, Mączyński M. 5-Amino-3-methyl-4-isoxazolecarboxylic acid hydrazide derivatives with in vitro immunomodulatory activities. Chem. Biol. Drug Des. 2017; 89: 705–713. DOI: 10.1111/cbdd.12892

35. Chaudhary PM, Eby MT, Jasmin A, Kumar A, Liu L, Hood L. Activation of the NF-kappa B pathway by caspase 8 and its homologs. Oncogene 2000; 19: 4451–4460. DOI: 10.1038/sj.onc.1203812
36. Liu F, Bardhan K, Yang D, Thangaraju M, Ganapathy V, Waller JL, Liles GB, Lee JR, Liu K. NF-κB directly regulates Fas transcription to modulate Fas-mediated apoptosis and tumor suppression. J. Biol. Chem. 2012; 282: 25530–25540. DOI: 10.1074/jbc.M112.356279
37. Mohan S, Abdelwahab SI, Kamalidehghan B, Syam S, May KS, Harmal NSM, Shafifiyaz N. Involvement of NF-κB and Bcl2/Bax signaling pathways in the apoptosis of MCF7 cells induced by a xanthone compound Pyranocycloartobiloxanthone A. Phytomedicine 2012; 19: 1007–1015. DOI: 10.1016/j.phymed.2012.05.012