SYNTHESIS AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF \{1-ARYL-4-CHLORO-1H-IMIDAZOLE-5-YL\} METHYL[THIO\}ALKANE CARBOXYLIC ACIDS

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This study is devoted to development of the optimal conditions for synthesis and the study of some "structure – antioxidant activity" regularities of \{1-arylimidazole-5-yl\}methylthioalkane carboxylic acids, which structural analogues have found an application as medicinal products with a wide range of biological activities. The methodology of interaction between 4-chloro-5-chloromethylimidazoles with thioglycolic and thiopropionic acids has been used to obtain these compounds. Selection of the optimal reaction conditions has allowed to obtain target compounds in a dry dimethylformamide in the presence of potash at 50°C with yields of 75-82%. The compounds synthesized are high-melting crystalline substances that dissolve well in polar organic solvents and aqueous alkaline solutions. Their composition and structure have been confirmed by the results of elemental analysis and measurement data of IR-, 1H NMR- and chromatography mass-spectra. The study of the compounds synthesized has been conducted in vitro on biological samples. The antioxidant activity has been determined by the inhibition value of the ascorbate-dependent endogenous lipid peroxidation rate in rats' liver found by the concentration of one of the final products of free-radical lipid oxidation processes – malonaldehyde in the test sample. The results of the biological activity screening of the compounds synthesized show that all imidazole derivatives studied in the final concentration ranges of 10⁻³-10⁻¹ M exhibit a high antioxidant action in the system in vitro. It has been found that the value of the antioxidant activity is influenced by the nature and position of the substituent in position 1 of imidazole. In particular, the presence of electron-acceptor substituents in the aryl fragment decreases the molecule activity in comparison with electron-donor substituents, wherein increase of the methylene groups quantity in the carboxyalkylthiol fragment does not significantly impact the antioxidant effect of the compounds synthesized.

The process of free-radical lipid oxidation (FRLO) plays a significant role in development of the most diseases of the liver, cardiovascular, respiratory and nervous systems [1, 16]. Antioxidants are widely used to normalize the basic organism functions as a part of complex therapy of such diseases [7]. It should be noted that at present there is insufficient amount of medicines with the antioxidant mechanism of action offered for clinical use, and those that are in use have many adverse effects and high toxicity [4]. Taking this into account, the search of compounds that are able to inhibit the FRLO processes effectively is of high interest nowadays.

Design and synthesis of new compounds with antioxidant properties is a subject of many studies as they are of high interest in prophylactics and therapy of many diseases that have FRLO in their pathogenesis [3, 9]. From this aspect the derivatives of imidazole are not left aside too; according to the literature data they are characterized by a wide spectrum of pharmacological properties, among which the antioxidant effect is particularly noteworthy [10, 11, 15].

Earlier we found the antioxidant activity in the series of \{1-phenyl-5-formyl-1H-imidazole-4-yl\}thioacetic acids and their derivatives [5, 6, 8, 13]. It was to be expected that the change in the position of the thioalkanecarboxylic acid fragment in the structure of the imidazole cycle would allow a much bigger quantity of potentially active compounds with the antioxidant action, and also give an opportunity to approach to the solution of the topical problem of pharmaceutical chemistry – establishing of the structure – activity relationship. With this purpose we have synthesized \{1-arylimidazole-5-yl\}methylthioalkanecarboxylic acids (2a-g) and carried out the screening of their antioxidant properties.

The reaction of easily available 4-chloro-5-chloromethylimidazoles (1a-d) [12] with thioglycolic and thiopropionic acids, which occurs selectively at 50°C in a dry DMFA in the presence of potash, has been used to obtain compounds of such kind. The reaction gives the target compounds (2a-g) with yields of 75-82%.

The compounds (2a-g) synthesized (Tab. 1, 2) are light-yellow, high-melting crystalline compounds, readily soluble in polar organic solvents and aqueous alkaline solutions. Their composition and structure were confirmed by elemental analysis and by the results of IR-, 1H NMR and chromatography mass-spectra measurements. IR-spectra, in particular, are characterized...
by intensive absorption bands of carbonyl groups at 1675-1685 cm\(^{-1}\) and by a wide absorption range (2430-2830 cm\(^{-1}\)) of carboxyl groups, and it indicates a dimeric character of the acids obtained in their solid state. In \(^1\)H NMR spectra of all compounds the most illustrative is the \(^1\)H-2 proton singlets of the imidazole cycle at 7.83-7.95 ppm and the singlets of methylene groups bound with the imidazole cycle at 3.72-3.85 ppm. In their turn, the methylene protons of thiocarboxylic acid fragments are visible as singlets at 3.17 ppm, and those of thioproionic acid – as triplets in the range of 2.30-2.35 and 2.49-2.54 ppm.

**Experimental Part (Chemistry)**

IR-spectra of the compounds synthesized were recorded on a UR-20 spectrophotometer in KBr tablets. \(^1\)H NMR spectra were recorded on a Varian-Mercu-

| Compound | IR-spectrum, cm\(^{-1}\) | \(^1\)H NMR, \(\delta\), ppm |
|----------|----------------------|----------------------|
| 2a       | 1685 2430-2850       | 3.17 s (2H, CH\(_2\)), 3.82 s (2H, CH\(_2\)), 7.36-7.43 m (2H\(_{\text{arom}}\)), 7.60-7.65 m (2H\(_{\text{arom}}\)), 7.87 c (1H, H\(_2\)imidazole), 12.55 brs. (1H, COOH) |
| 2b       | 1680 2450-2840       | 3.17 s (2H, CH\(_2\)), 3.85 s (2H, CH\(_2\)), 7.57-7.63 m (4H\(_{\text{arom}}\)), 7.90 s (1H, H\(_2\)imidazole), 12.60 brs. (1H, COOH) |
| 2c       | 1680 2450-2820       | 2.38 s (3H, CH\(_3\)), 3.17 s (2H, CH\(_2\)), 3.82 s (2H, CH\(_2\)), 7.36 d (2H\(_{\text{arom}}\), J 8.0 Hz), 7.41 d (2H\(_{\text{arom}}\), J 8.0 Hz), 7.83 s (1H, H\(_2\)imidazole), 12.54 brs. (1H, COOH) |
| 2d       | 1675 2435-2830       | 2.34 t (2H, CH\(_2\), J 6.8 Hz), 2.54 t (2H, CH\(_2\), J 6.8 Hz), 3.75 s (2H, CH\(_2\)), 7.49-7.55 m (5H\(_{\text{arom}}\), 7.87 s (1H, H\(_2\)imidazole), 12.24 s. (1H, COOH) |
| 2e       | 1680 2440-2835       | 2.32 t (2H, CH\(_2\), J 6.8 Hz), 2.50 t (2H, CH\(_2\), J 6.8 Hz), 3.77 s (2H, CH\(_2\)), 7.40-7.48 m (2H\(_{\text{arom}}\), 7.62-7.69 m (2H\(_{\text{arom}}\)), 7.90 s (1H, H\(_2\)imidazole), 12.28 brs. (1H, COOH) |
| 2f       | 1680 2435-2850       | 2.35 t (2H, CH\(_2\), J 6.8 Hz), 2.49 t (2H, CH\(_2\), J 6.8 Hz), 3.76 s (2H, CH\(_2\)), 7.56 d (2H\(_{\text{arom}}\), J 8.0 Hz), 7.64 d (2H\(_{\text{arom}}\), J 8.0 Hz), 7.94 s (1H, H\(_2\)imidazole), 12.20 brs. (1H, COOH) |
| 2g       | 1685 2445-2840       | 2.30 t (2H, CH\(_2\), J 6.8 Hz), 2.38 s (3H, CH\(_3\)), 2.53 t (2H, CH\(_2\), J 6.8 Hz), 3.72 s (2H, CH\(_2\)), 7.36 d (2H\(_{\text{arom}}\), J 7.0 Hz), 7.42 d (2H\(_{\text{arom}}\), J 7.0 Hz), 7.95 s (1H, H\(_2\)imidazole), 12.40 brs. (1H, COOH) |
The antioxidant activity of \[\{(1\text{-}aryl\text{-}4\text{-}chloro\text{-}1H\text{-}imidazole\text{-}5\text{-}yl)methyl\}thio\text{alkanecarboxylic acids in vitro}\]

| Compound | Concentration, mole/l | 10^{-1} | 5 \times 10^{-2} | 10^{-2} | 5 \times 10^{-3} | 10^{-3} |
|----------|----------------------|---------|----------------|--------|----------------|--------|
|          | MA, \(\mu\text{mole/g of the tissue}\) | AOA, % | MA, \(\mu\text{mole/g of the tissue}\) | AOA, % | MA, \(\mu\text{mole/g of the tissue}\) | AOA, % | MA, \(\mu\text{mole/g of the tissue}\) | AOA, % |
| 2a       | 45.68±0.19*          | 60.8    | 46.32±0.12*    | 60.2   | 44.39±0.12*    | 61.9   | 46.19±0.07*    | 60.3   | 45.16±0.12*    | 61.2   |
| 2b       | 66.26±0.25*          | 43.1    | 53.14±0.19*    | 54.4   | 44.78±0.32*    | 61.5   | 43.36±0.19*    | 62.8   | 50.31±0.31*    | 56.8   |
| 2c       | 50.44±0.19*          | 56.7    | 53.01±0.07*    | 54.5   | 44.39±0.12*    | 61.9   | 49.79±0.21*    | 57.2   | 45.42±0.19*    | 61.0   |
| Control 1| 100.36±0.37          | 100.36±0.37 | 100.36±0.37 | 100.36±0.37 | 100.36±0.37 | 100.36±0.37 | 100.36±0.37 | 100.36±0.37 | 100.36±0.37 |
| 2d       | 24.19±0.07*          | 59.0    | 16.73±0.07*    | 71.6   | 14.02±0.07*    | 76.2   | 13.90±0.12*    | 76.4   | 16.60±0.12*    | 71.9   |
| 2e       | 28.95±0.12*          | 50.9    | 20.07±0.12*    | 66.0   | 16.98±0.32*    | 71.2   | 29.34±0.12*    | 50.3   | 20.46±0.12*    | 65.3   |
| 2f       | 23.93±0.12*          | 59.4    | 24.58±0.19*    | 58.3   | 20.84±0.24*    | 64.7   | 35.13±0.12*    | 40.5   | 35.13±0.12*    | 40.5   |
| 2g       | 17.37±0.12*          | 70.5    | 19.56±0.07*    | 66.8   | 23.03±0.14*    | 61.0   | 26.25±0.12*    | 55.5   | 24.70±0.12*    | 58.1   |
| Control 2| 59.06±0.12           | 59.06±0.12 | 59.06±0.12 | 59.06±0.12 | 59.06±0.12 | 59.06±0.12 | 59.06±0.12 | 59.06±0.12 |
| Thiotriazole| 70.64±0.56*       | 38.5    | 73.98±0.19*    | 35.6   | 77.33±0.25*    | 32.7   | 76.43±0.24*    | 33.5   | 79.13±0.12*    | 31.2   |
| Control 4| 115.03±0.24          | 115.03±0.24 | 115.03±0.24 | 115.03±0.24 | 115.03±0.24 | 115.03±0.24 | 115.03±0.24 | 115.03±0.24 |

* = valid in relation to control \((p \leq 0.05)\)

Results and Discussion

The results of the antioxidant activity screening of the compounds synthesized \textit{in vitro} (Tab. 3) show that all compounds are able to inhibit Fe^{2+}-ascorbate initiated FRLO in these conditions in the final concentration ranges of \(10^{-3}-10^{-1}\) M studied. Thus, the degree of Fe^{2+}-ascorbate initiated FRLO inhibition \textit{in vitro} of all original compounds synthesized was higher than the antioxidant activity of thiotriazoline in the same final concentrations. The antioxidant activity of thiotriazole in the specified range of final concentrations \textit{in vitro} varied between 31.32% and 38.59% and was the highest with the final drug concentration of \(10^{-1}\) M. With the same final concentration the highest inhibiting effect on the initiated FRLO was shown by compound 2g (the degree of FRLO inhibition was 70.59%). Most compounds (2a, 2c, 2e, 2f) have shown the highest antioxidant activity \textit{in vitro} in the concentrations of \(10^{-2}\) M. The highest antioxidant effect \textit{in vitro} was recorded for compound 2d in the range of the final concentrations of \(5 \times 10^{-3}-10^{-2}\) M: the degree of FRLO inhibition was 76.47-76.25%. On average, it is 43% higher than the results shown by thiotriazole in the same range of the final concentrations, and 37% higher than the maximum effect of thiotriazole recorded \textit{in vitro}.

Analysis of the data obtained shows that the value of the antioxidant activity is influenced by the nature and position of the substituent in position 1 of imidazole. In particular, the presence of electron-acceptor substituents in the aryl fragment decreases the molecule activity in comparison with electron-donor substituents, wherein increase of the methylene groups quantity in the carboxyalkylthiol fragment does not significantly impact the antioxidant effect of the compounds synthesized.

CONCLUSIONS

1. By interaction of 5-chloromethylimidazoles with thioglycolic and thiopropionic acids new \(\{(1\text{-}aryl\text{-}4-
chloro-1H-imidazole-5-yl)methyl[thio]alkanecarboxylic acids have been synthesized.

2. All derivatives of imidazole studied in the range of concentrations of 10^{-3}\text{ to } 10^{-1}\text{ M} show a high antioxidant activity in the system in vitro. The highest activity was recorded for compound 2d in the final concentration of 5\times10^{-3}\text{ M}.

3. Increase of the methylene groups quantity in the carboxyalkythiol fragment does not significantly affect the antioxidant effect of the compounds synthesized.

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СИНТЕЗ И ОЦЕНКА АНТИОКСИДАНТНОЙ АКТИВНОСТИ \{(1-АРИЛ-4-ХЛОР-1H-ИМИДАЗОЛ-5-ИЛ)МЕТИЛТИО}АЛКАНКАРБОНОВЫХ КИСЛОТ

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Ключевые слова: синтез; имидазол; \{(1-арил-5-формил-1H-имидазол-4-ил)тио\}уксусные кислоты; \{(1-арил-4-хлоро-1Н-имидазол-5-ил)метил\}титанкарбоновые кислоты; антиоксидантная активность

Исследование посвящено разработке оптимальных условий синтеза и изучению некоторых закономерностей «структура-антиоксидантная активность» \{(1-арилимидазол-5-ил)метилтио\}алканкарбоновых кислот, структурные аналоги которых нашли применение в качестве лекарственных средств с широким спектром биологического действия. Для получения указанных соединений использована методология, которая заключается во взаимодействии 4-хлор-5-хлорметилимидазолов с тиогликолевой и тиопропановой кислотами. Подбор оптимальных условий протекания реакции позволил получить целевые соединения в сухом ДМФА в присутствии поташа при 50°С с выходами 75-82%. Синтезированные соединения – высокоплавкие кристаллические вещества, хорошо растворимые в полярных органических растворителях и водных растворах щелочей. Их состав и структура подтверждены результатами элементного анализа и данными измерений ИК-, ЯМР 1H- и хроматомасс-спектров. Исследование синтезированных соединений проводили in vitro на биологических образцах. Антиоксидантную активность определяли по величине ингибирования скорости аскорбат-зависимого перекисного окисления эндогенных липидов в печени крыс, которую устанавливали по концентрации одного из конечных продуктов процессов СРОЛ – малонового альдегида в исследуемом образце. Результаты скрининга биологической активности синтезированных соединений свидетельствуют о том, что все исследованные производные имидазола в диапазоне конечных концентраций 10^-3-10^-1 М проявляют высокое антиоксидантное действие в системе in vitro. Установлено, что на величину антиоксидантной активности влияет характер и положение заместителя в положении 1 имидазола. В частности, присутствие в арильном фрагменте электронодонорных заместителей снижает активность по сравнению с электроноакцепторными. При этом увеличение количества метиленовых групп в карбоксилкетионном фрагменте существенно не влияет на антиоксидантный эффект синтезированных соединений.