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## SYNTHESIS OF NEW INDOLE DIMERS WITH TRIAZOLE RING

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### Abstract:

Six new indole derivatives with triazole rings were obtained. The four synthesized compounds were indole dimers linked by two 1,2,3-triazole rings and the other two compounds are combined of two different derivatives of indole linked by one 1,2,3-triazole ring. All new dimmers were obtained by CuACC procedure and characterized by spectral methods.

### Keywords:

*indole; gramine; click chemistry; dimers; spectroscopic analysis*

### Introduction

Indole (2,3-benzopyrrole) (Fig. 1.) is a heterocyclic chemical compound combined with two condensed rings: five-membered pyrrole and six-membered benzene rings. Its structure occurs in many compounds present in the tissues of living organisms, both plant and animal. Because of its high biological activity, the indole scaffold has been recognized, over the years, as a model for the synthesis of compounds with various physiological activities that may be considered as potential drugs. They reveal antioxidant, anticancer, anti-inflammatory, antiviral, antibacterial properties [1]. A large group of derivatives of indole are indole alkaloids. They are compounds of plant origin showing a wide range of biological properties. For example, derivatives of lysergic acid, called ergot alkaloids, are very toxic, but they exhibit oxytocic effect in small doses. A commonly known psychotropic agent – *N,N*-diethylamide of lysergic acid is also one of the ergot alkaloids containing indole structure. The strychnine in *Strychnos nux vomica* seeds is one of the most potent poisons that paralyses the central nervous system. Despite its toxicity, in small amounts, it can be used as an analeptic and treat respiratory disorders. To a group of indole alkaloids also belongs gramine – tertiary amine also called donaxine [1-6]. Gramine is used as a starting compound to synthesise new indole analogues. Gramine and its derivatives induce relaxation of bronchial smooth muscles, increase blood pressure, relieve the symptoms of bronchitis and asthma, play an important role in the metabolism of amino acids and affect redox processes. They are also used as anti-corrosion, anti-fouling, antibacterial, antiviral and anticancer agents [6-8]. Fig. 1. presents structures of indole and its derivatives.

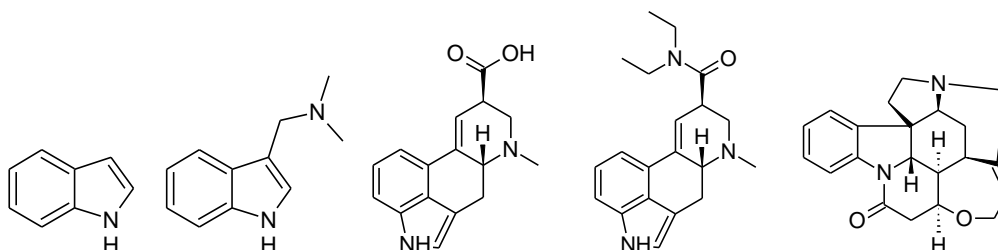


Fig. 1. The structures of indole, gramine, lysergic acid, LSD and strychnine  
Source: own elaboration

Definitely noteworthy is indole analogue of plant origin – *1H*-indole-3-methanol, commonly known as indole-3-carbinol (I3C). This compound is found in cruciferous vegetables such as cabbage, cauliflower, broccoli, cucurbit and Brussels sprouts. I3C is relatively unstable, and in acidic conditions of the stomach, it is converted to oligomeric structures, mostly dimers (DIM) and trimers (CTr, ICZ) (Fig. 2.). The literature data shows that people whose diets are rich in cruciferous vegetables are less exposed to cancer.

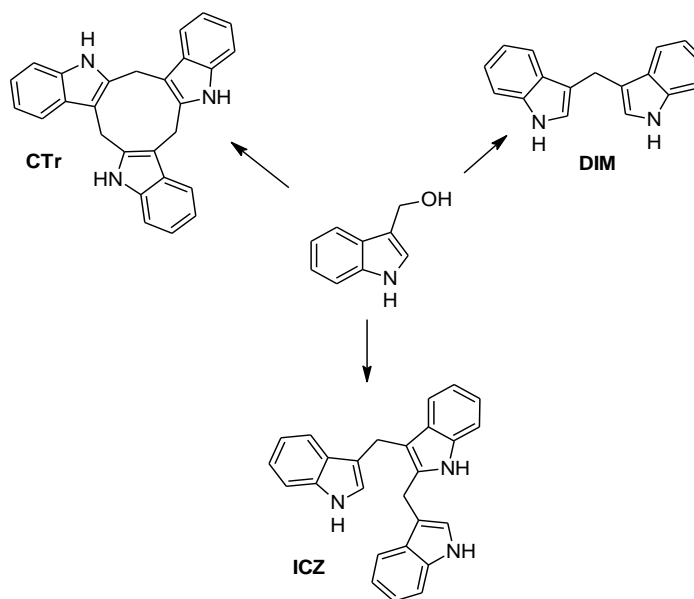


Fig. 2. The structures of indole-3-carbinol (I3C) and its oligomers: 3,3'-diindolylmethane (DIM), indole(2,3b)carbazole (ICZ) and cyclic trimer (CTr)  
Source: own elaboration

DIM exhibits higher physiological activity than its monomer. That is why it is suspected that forming dimers by I3C determines its anticancer properties. I3C and DIM induce apoptosis of prostate, oral, colorectal, pancreatic, liver, gastric, cervix and colon cancer cells. They also inhibit the growth of breast cancer cells of the MDA-MB-231, MDA-MB-435 and MCF10CA1a lines and prostate cancer (PC3). DIM has protective effects against liver injury, cardiac-inflammatory responses and renal fibrosis [1, 6, 9]. Literature data show that 3,3'-diindolylmethane with anticancer activity has a strong antioxidant effect. This dimer attenuates toxicity of doxorubicin (DOX) – a commonly used cytostatic agent. Like most chemotherapeutic agents, doxorubicin induces apoptosis of cancer cells,

but unfortunately also has a cytotoxic effect on healthy normal cells, for example, causing oxidative stress. Recent studies prove that DIM significantly reduces the level of free radicals and lipid peroxidation, which weakens DOX-induced oxidative stress. Furthermore, oral consumption of I3C dimer stimulates the Nrf2-mediated activation of antioxidant response element (ARE), an intrinsic defence mechanism against oxidative stress. DIM was tested for anti-inflammatory activity, and it turned out that this indole dimer reduces the amount of pro-inflammatory cytokines [10, 11].

In our previous work, we synthesized a series of 3-ethoxymethylindole dimers linked by aliphatic chains (Fig. 3.). They were tested for biological activity. The derivative with propyl linker showed high cytoprotective properties against free radicals induced oxidative haemolysis [12].

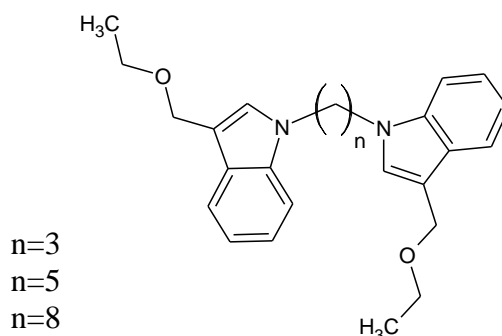


Fig. 3. Structures of 3-ethoxymethylindole dimers  
Source: own elaboration

Click chemistry is a very useful tool for combining various molecules together. It takes place in a short time and under mild conditions. This method is based on reaction between azide and terminal alkyne and leads to formation of very stable 1,2,3-triazole ring. Thanks to the participations of Cu (I) ions the reaction is regioselective, which results in a synthesis of di-substituted (at the N1 and C4 positions) triazoles. This method is known as copper (I) - catalyzed Huisgen 1,3-dipolar cycloaddition (CuAAC) and is widely used for the synthesis of compounds having potential biological activity [6, 13, 14].

## Experimental

3-propargyloxymethylindol (2) was synthesized according to [12].

Diazoalkanes (3-6) were synthesized according to [15].

Compound 11 was synthesized according to [6].

### General procedure for the preparation of compounds 7-10

3-propargyloxymethylindole (1 mmol) was dissolved in THF (2 mL). Then an appropriate azide (0.5 mmol) was added.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (5 mg, catalytic amount) and sodium ascorbate (13 mg, catalytic amount) in 1 mL distilled water were added. The mixture was stirred on a magnetic stirrer at room temperature. Subsequent catalyst portions were added until the aqueous layer of the reaction mixture turned bluish-green. The reaction was monitored by TLC (PhMe:EtOAc 5:1). A brown solid precipitated as the reaction mixture was stirred. The precipitate was filtered off under reduced

pressure, then washed with distilled water and diethyl ether. The precipitate was dried at room temperature.

#### **Preparation of *N*-acehyl-3-azidomethylindole (12)**

*N*-acetyl-3-carbinol (0.5 mmol) was dissolved in DMF (2 mL). Diluted in toluene (1 mL), diphenyl phosphoryl azide (1 mmol) was added. The reaction mixture was cooled to 0°C, and 1.8-diazabicyclo[5.4.0]undec-7-ene (1 mmol) was dropped to the reaction mixture. The reaction mixture was stirred for 2 h in 0°C. The reaction was monitored by TLC (PhMe:EtOAc 5:1). The reaction was quenched with 5 mL of distilled water. The reaction mixture was then extracted with ethyl acetate (3 x 10 mL). Then the organic layer was washed with distilled water (3 x 10 mL), washed with brine (50 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The organic layer was concentrated under reduced pressure. Crude product was purified over column chromatography using PhMe:EtOAc 50:1 as an eluent. The yellow oil was obtained.

#### **Synthesis of *N*-propargyl-3-etoxyethylindole (13)**

To 3-etoxyethylindole (0.7 mmol) dissolved in DMF (2 mL), crushed pellets of KOH was added (60 mg). The propargyl bromide (80% solution in toluene, 0.9 mmol) was dropped to the reaction mixture. The reaction mixture was stirred at room temperature for 24 hours. The reaction was monitored by TLC (PhMe:EtOAc 5:1). The reaction mixture was then extracted with diethyl ether (3 x 10 mL). Then the organic layer was washed with distilled water (3 x 10 mL), washed with brine (50 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The organic layer was concentrated under reduced pressure. The crude product was purified using PhMe:EtOAc 50:1 as an eluent over column chromatography. Brown oil was obtained.

#### **General procedure for the preparation of compounds 14 and 15**

3-propargyloxymethylindol (0.4 mmol, for the compound 14) or *N*-propargyl-3-etoxyethylindole (0.4 mmol, for the compound 15) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). *N*-acetyl-3-azidomethylindole was added. CuSO<sub>4</sub>·5H<sub>2</sub>O (5 mg, catalytic amount) and sodium ascorbate (13 mg, catalytic amount) in 1 mL distilled water were added. The mixture was stirred on a magnetic stirrer at room temperature. Subsequent catalyst portions were added until the aqueous layer of reaction mixture turned bluish-green. The reaction was monitored by TLC (PhMe:EtOAc 1:1). The reaction mixture was extracted with ethyl acetate (3 x 10 mL). Then the organic layer was washed with distilled water (3 x 10 mL), washed with brine (50 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The organic layer was concentrated under reduced pressure. Brown oil was obtained. The crude product was purified over column chromatography using gradient elution, starting from eluent toluene/ethyl acetate 5:1, finishing on a 1:1 ratio – in case of compound 14, for compound 15 chloroform/ethyl acetate was used as eluent, starting from 5:1 and finishing on a 1:1 ratio. The thick, brown oil was obtained.



## Results and discussion

The structures of newly obtained compounds were confirmed by spectral methods such as FT-IR spectroscopy  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR. The most characteristic absorption bands are shown on the spectrum below (Fig. 4.). We can see the band with the maximum at  $3395.76\text{ cm}^{-1}$  corresponding to the N-H bond from the indole amine group. A small band at  $3055.95\text{ cm}^{-1}$  is related to the carbon-hydrogen bond from the triazole ring. We can also see bands with the maximum at  $2927.98\text{ cm}^{-1}$  and  $2855.57\text{ cm}^{-1}$  derived from an aliphatic chain.

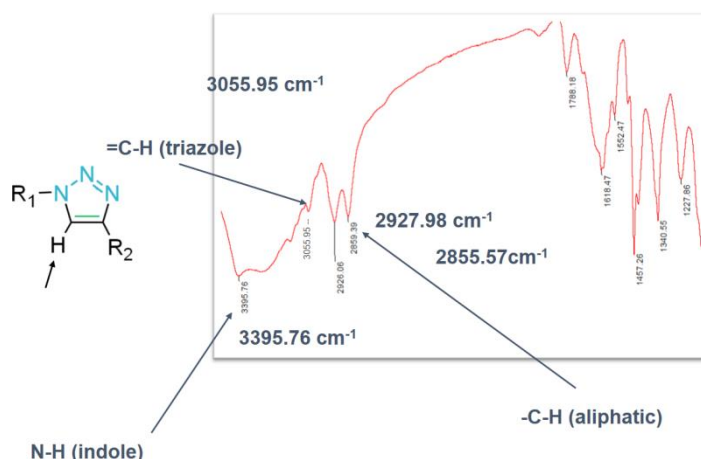


Fig. 4. FT-IR spectrum of the compound 9

Source: own elaboration

Nuclear Magnetic Resonance also confirms structures of obtained dimers. The  $^1\text{H}$  NMR spectra of compounds 8 and 14 (Figs. 5. and 6.) show strong signals from two protons belonging to the amine groups of two indole moieties. We can also see signals from triazole rings at about 11 ppm. In the range of 7.5 ppm to 6.5 ppm occurs signals from other protons of indole and at lower values of chemical shift – signals from methylene groups from an aliphatic chain. The number of signals on the  $^{13}\text{C}$  NMR spectrum corresponds to the number of carbon atoms of the dimer. The  $^{13}\text{C}$  NMR spectra of compounds 8 and 14 show signals from C4 atom from triazole ring at about 138 ppm, signals in the range of 130 – 110 ppm belong to the carbon atoms from aromatic rings. Signals at the lower chemical shift values are correlated to the aliphatic carbon chain.

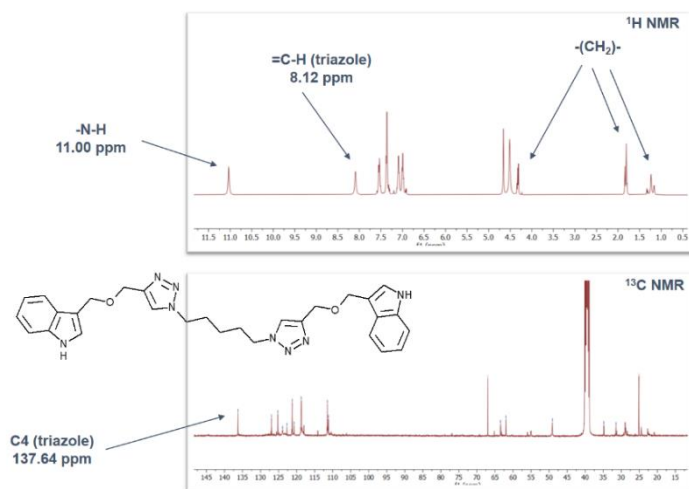


Fig. 5.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of compound 8  
Source: own elaboration

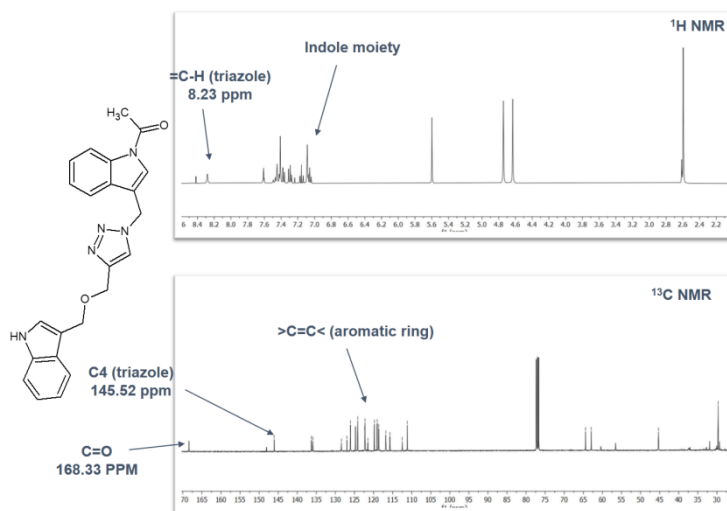


Fig. 6.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of compound 14  
Source: own elaboration

For compounds 14 and 15, the ESI-MS spectra were recorded. For derivative 14, the dominant ion at  $m/z = 422$  corresponds to the protonated ion  $[399 + \text{Na}]^+$ , where 399 refers to the molecular mass of compound 14 (Fig. 7.).

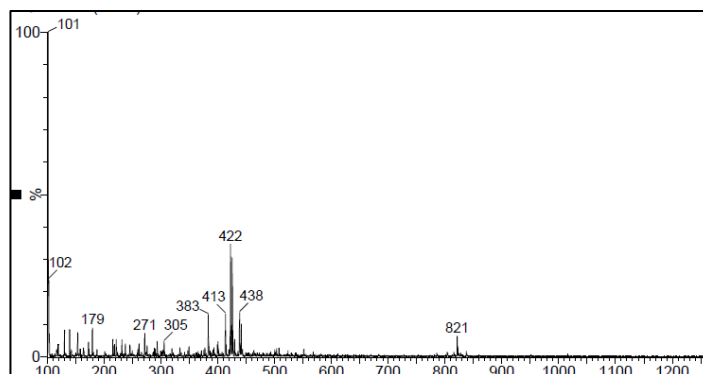


Fig. 7. ESI-MS spectrum of compound 14

Source: own elaboration

Schemes of synthesis compounds 7-10, 14 and 15 are shown in the pictures below (Figs. 8. and 9.).

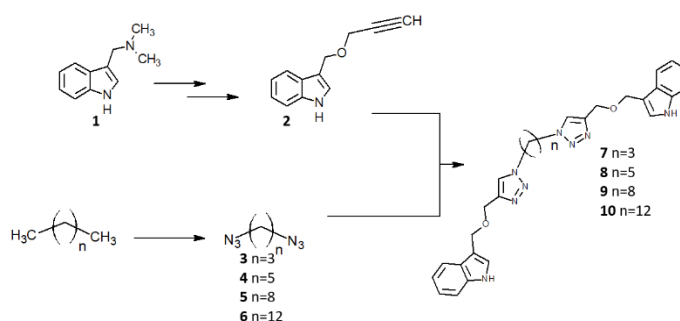


Fig. 8. The synthesis scheme of compounds 7-10

Source: own elaboration

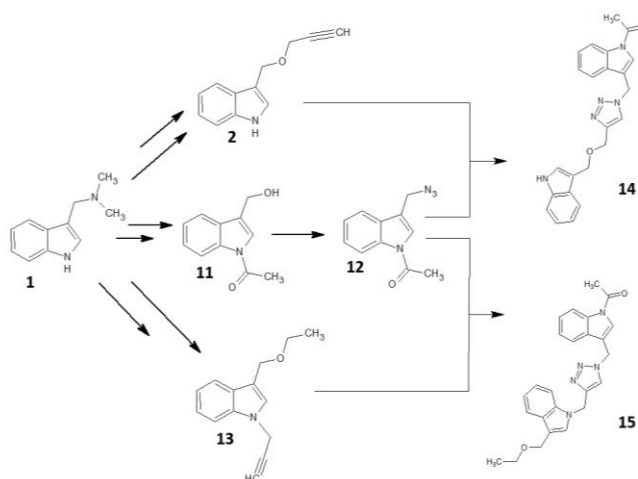


Fig. 9. The synthesis scheme of compounds 14 and 15

Source: own elaboration

## Theoretical calculations

In order to investigate the molecular geometry, energy and dipole moments of the derivatives 7-10, calculations were carried out using the method based on the density functional theory (DFT) using the B3LYP functional in the 6-311G database (d, p). The results of the calculations are shown in the table below (Tab. 1.).

Tab. 1. The energy of compounds 7-10 estimated by B3LYP/6-311G (d, p) calculations

Compound	Energy [a.u.]
7	-1633.98905843
8	-1712.61519318
9	-1830.54929096
10	-1987.79516837

Source: own calculations

The data presented in the table shows that the energy depends on the length of the alkyl chain. The longer the chain, the lower the energy. It is related to the symmetry of the molecules and the possibility for the molecules to adopt different conformations. Derivatives with longer carbon chains are more thermodynamically stable. The structures of derivatives with propyl and pentyl linkers (7 and 8) are linear, whereas compounds with octyl and dodecyl linkers (9 and 10) are folded. The optimized structures of compounds 7, 8, 9 and 10 are presented in the figure below (Fig. 10.).

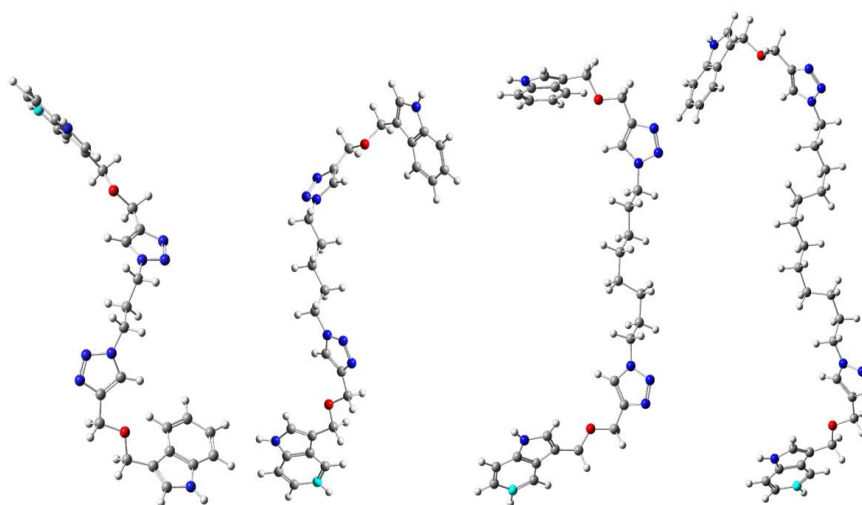


Fig. 10. Structures of compounds 7, 8, 9 and 10 calculated by B3LYP / 6-311G (d, p) method  
Source: own elaboration

## Conclusion

The click chemistry turned out to be a very useful method of synthesising indole dimers. In addition to the indole moiety, the products molecules also contain one or two 1,2,3-triazole rings in their structure. The spectral methods confirmed the structures of these dimers. The theoretical calculations showed that the most stable is derivative with the longer aliphatic chain. Newly obtained triazole indole derivatives may be potential compounds with a therapeutic effect.

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# OVERVIEW OF PLANT CELL PROTEASES ON THE EXAMPLE OF *ARABIDOPSIS THALIANA*

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## **Abstract:**

The enzymes that carry out the reaction that breaks a peptide bond are mainly hydrolytic enzymes are referred to as proteases. Plant proteases play a critical role in nearly all aspects of plant life. The plant genome encodes proteases that act on many levels, in development, homeostasis, abiotic and biotic stress. Their activity is strictly regulated. In this article, I gave a general overview of plant proteases using *Arabidopsis thaliana* as an example.

## **Keywords:**

*protease; proteolysis; A.thaliana*

## **Introduction**

There are three distinct systems of enzymatic degradation of cell proteins in plant cells. Most proteins are degraded by the 26S proteasomes (UPS system), which is involved in the hydrolysis of proteins that have undergone the ubiquitination process [1]. Independently of it, several variants of the autophagy process function in plant cells, which consist in digesting cell fragments in the lytic vacuole [2]. Autonomous organelle proteases - this is the third proteolytic system of plant cells, functioning in certain cell compartments, such as chloroplasts, peroxisomes, mitochondria, and the lumen of the endoplasmic reticulum.

## **Ubiquitin - 26S proteasome (UPS)**

UPS is a cytoplasmic system for the degradation of short-lived proteins. Relevant components of the UPS system recognize specific protein sequence motifs or elements of the superior structure of proteins and ubiquitinate the proteins that contain them. Signals directing to the polyubiquitination pathway include the N-terminal amino acid residue [3], one of the internal lysine residues [4], PEST sequence [5], or abnormal secondary or tertiary structure [4]. Several/several dozen ubiquitin molecules are covalently joined to the proteins targeted at the UPS degradation pathway. Substrate polyubiquitination takes place in the course of a multistage reaction carried out sequentially by enzymes belonging to three groups: enzymes that activate ubiquitin in an ATP-dependent manner

(E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) that recognize the target protein and catalyze the transfer of activated ubiquitin from E2 to it [6]. The polyubiquitinated proteins are recognized by the relevant structural components of the 26S proteasomes and degraded internally.

## **Lytic vacuoles**

The functioning of vacuolar proteases combines elements of metabolic turnover control with elements of substrate quality control protein. There are no data that describe the motifs that render protein molecules susceptible to degradation by vacuolar proteases. In some plant species in the seed protein storage vacuoles during embryogenesis, proteases have been identified that are involved in the processing of precursor forms of storage proteins to mature forms [7].

## **Autophagy**

The degradation of long-lived proteins proceeds in a manner non-selective, involving mainly several variants of autophagic processes, i.e. mass degradation processes aimed mainly at recovering nutrients, getting rid of interprotein aggregates, and responding to various stresses. Thanks to permanent autophagic processes in the cell the physiological concentration of nutrients is maintained, and increasing such activity helps to remove dysfunctional organelles [8]. There are at least four autophagy pathways in plant cells: macroautophagy, microautophagy, CVT (cytoplasm-vacuole transport), and micropexophagy [2]. Macroautophagy involves the surrounding of a large fragment of the cytoplasm with a double membrane and the formation of an autophagosomal vesicle, the membrane of which is then fused with the central lytic vacuole and the exposed load of the autophagosome, now referred to as the autophagic body, is degraded [9]. The CVT pathway is very similar, the difference from macroautophagy is that in the autophagosome (very small in this variant of autophagy), single proteins are captured [2]. In the microautophagy variant, a small fragment of the cytoplasm is introduced into the lumen of the lytic vacuole in the form of a vesicle formed by local invagination of the tonoplast, and then the vesicle is released into the vacuole or lysosome and its charge is degraded thereby numerous hydrolytic enzymes (including proteases) [8].

## **Mitochondrial proteases**

The main components of the mitochondrial proteolytic system are highly conserved in evolution. They are divided into three groups: processing proteases, ATP-dependent proteases, and oligopeptides [10]. In *Arabidopsis*, 77% of the mitochondrial proteins encoded by the nuclear genome have targeting sequences that are cleaved by mitochondrial-processing peptidase (MPP) upon import of proteins into the mitochondria [11].

The localization of plant MPPs is in the inner mitochondrial membrane and they are part of the cytochrome bc<sub>1</sub> complex of the respiratory chain [12]. Rhomboids are processing serine proteases involved in the intramembrane proteolysis of membrane-bound substrates within their domain. transmembrane, which results in the release of biologically active proteins into the intermembrane space [13]. Only one *A. thaliana* rhomboid protease has been identified directed to mitochondria - AtRBL12 (*Arabidopsis thaliana* rhomboid-like) belonging to the subfamily AtPARL [14].

Three types of ATP-dependent proteases Lon, Clp, and FtsH have been identified in plant mitochondria [15], the first two of which are serine proteases and FtsH are metalloproteases. Their characteristic feature is the presence of the ATPase domain (AAA + domain) acting as a chaperone. Clp proteases consist of a proteolytic core formed by ClpP2 and a ring of chaperone subunits (ClpX1-3) [16].

FtsH is proteases having a characteristic catalytic center for Zn<sup>2+</sup> dependent metalloproteases and constituting a single protein. Four FtsH proteases are targeted to *A.thaliana* mitochondria: AtFtsH3, AtFtsH4, AtFtsH10 and AtFtsH11 [17]. In *Arabidopsis* mitochondria, we also find products of two genes encoding ATP-dependent Lon proteases - AtLON1 and AtLON4 [18], in which the protease domain (LON-C) and the AAA + domain are found in the linear structure. PreP (presequence protease) oligopeptides that degrade mitochondrial targeting sequences have been identified in the mitochondrial matrix [19]. In *A. thaliana*, two highly homologous, nuclear-encoded PreP isoforms are distinguished: AtPreP1 and AtPreP2 [20]. Both isoforms have an inverted Zn<sup>2+</sup> binding motif and belong to the family of metalloproteases [21].

## Peroxisomal proteases

The best known peroxisomal proteases include AtLon2 and AtDeg15. AtLon2 is a multifunctional protein that degrades misfolded and non-complexed proteins - partners of the peroxisomal matrix protein, as well as protein aggregates [22]. It can also act as a chaperone [23]. AtDeg15 probably cuts off the N-terminal sequence PTS2 signaling [24] from cytoplasmic imported peroxisomal protein precursors [25].

## ER light protease

ER light proteins are synthesized on membrane-bound polysomes and cotranslationally directed to the ER through a translocation channel consisting of a complex Sec61 protein [26]. The newly synthesized polypeptide chain is transferred to the ER lumen in an elongated and unfolded state, while the folding process takes place inside the ER and takes place under the supervision of machinery catalytic. The abnormal course of the folding process is associated with the removal of such a protein by proteolytic degradation and takes place at the site of ER synthesis, therefore it is called the ERAD (ER-associated degradation) proteolytic process.

## Chloroplast proteases

Chloroplasts are organelles inside which protein degradation is catalyzed by its proteases. Chloroplast proteases were discovered thanks to the observation that during the in vitro incubation of chloroplasts isolated from pea leaves, some of the proteins underwent light and ATP-dependent degradation [27]. It has been shown that degradation in such an experimental system can also take place independently of ATP [27]. In *A. thaliana*, there is quite a wide variety of chloroplast proteins belonging to the Clp family (S14 according to MEROPS classification), which are orthologs of the corresponding *E. coli* Clp proteins. Sixteen genes coding for *E. coli* orthological proteins for DegP, DegQ, and DegS have been identified in the *A. thaliana* nuclear genome; they were named AtDEG1-



16 [28]. It has been experimentally proven that five of these genes - AtDEG1, 2, 5, 7, and 8 - encode proteins targeting chloroplasts. The *Arabidopsis thaliana* nuclear genome contains 12 protein-coding genes orthologous to FtsH relative to *E. coli* FtsH [29]. The following are most likely targeted to chloroplasts: AtFtsH1, 2, 5, 6, 7, 8, 9, 12, and AtFtsH11 targets both chloroplasts and mitochondria [30].

### Clp proteases

AtClp (*A. thaliana* proteases) are located in the chloroplast stroma; the exception is AtClpC1, which is a protein with a dual localization: steep + inner membrane of the chloroplast envelope [31]. Almost all AtClp proteins are encoded by nuclear genes and genes synthesized on cytoplasmic ribosomes in the form of precursor molecules. The exception is ClpP1 - this protein is encoded by the chloroplast genome and synthesized on the stroma ribosomes immediately in the mature form. The Clp *A. thaliana* holoenzyme is an oligomeric complex consisting of five types of proteins: AtClpP proteases, proteolytically inactive AtClpR proteins, chaperones belonging to the HSP100 family, AtClpS1 adapter protein and ClpT clusters of unknown function [32]. A single particle in total AtClp holocomplex is composed of eleven individual proteins. The proteolytic core is formed by the proteins AtClpP1 and AtClpR1-R4 in the form of a heptameric P ring.

Tab. 1. Data on genes encoding chloroplast AtClp proteases. The table was created from the gene data contained in the NCBI database. Gene length in base pairs

Proteases	Gene	Gene length
AtClpB3	<i>AtCLPB3</i>	4136
AtClpC1	<i>AtCLPC1</i>	4906
AtClpC2	<i>AtCLPC2</i>	4546
AtClpD	<i>AtCLPD</i>	4367
AtClpP1	<i>AtCLPP1</i>	2095
AtClpP3	<i>AtCLPP3</i>	1918
AtClpP4	<i>AtCLPP4</i>	1815
AtClpP5	<i>AtCLPP5</i>	2250
AtClpP6	<i>AtCLPP6</i>	2402
AtClpR1	<i>AtCLPR1</i>	3040
AtClpR2	<i>AtCLPR2</i>	2095
AtClpR3	<i>AtCLPR3</i>	2719
AtClpR4	<i>AtCLPR4</i>	3658
AtClpS1	<i>AtCLPS1</i>	1311
AtClpT1	<i>AtCLPT1</i>	2163
AtClpT2	<i>AtCLPT2</i>	1031

### Deg proteases

There are 16 Deg proteins present in the *A. thaliana* nuclear genome. Five of them - AtDEG1, 2, 5, 7, and 8 - are proteins targeting chloroplasts, AtDEG10 - a protein with mitochondrial localization, and AtDEG15 - a peroxisomal protein. The other nine AtDEG genes encode proteins whose localization has been determined only in silico, as both mitochondrial and chloroplast, or their localization could not be determined [24]. Most AtDEG genes have orthologies among other model plant species such as *Populus trichocarpa*, *Oryza sativa*, *Physcomitrella patens* and *Chlamydomonas reinhardtii* [28]. AtDeg are serine proteases that the MEROPS database places in the S1 family.

Tab. 2. Data on genes encoding chloroplast AtDeg proteases. The table was created from the gene data contained in the NCBI database. Gene length in base pairs

Proteases	Gene	Gene length
AtDeg1	<i>AtDEG1</i>	2535
AtDeg2	<i>AtDEG2</i>	4247
AtDeg5	<i>AtDEG5</i>	4266
AtDeg7	<i>AtDEG7</i>	2090
AtDeg8	<i>AtDEG8</i>	8908

### FtsH proteases

The products of the genes encoding chloroplast AtFtsH1, 2, 5, and 8 are proteins integrally associated with the thylakoid membrane [33], forming a heterooligomeric complex (called the AtFtsH heterocomplex) containing AtFtsH1/5 (type A AtFtsH) and AtFtsH2/8 (type B AtFtsH) [34]. Under normal conditions, FtsH2 is the most abundant chloroplast the isoform of AtFtsH, FtsH5 is the second most abundant, and FtsH8 and FtsH1 are represented by very small amounts [33]. In the primary structure of AtFtsH1, 2, 5, and 8 we find the AAA + domain, which contains the motifs A and B (involved in the binding and hydrolysis of ATP necessary for the unfolding of the substrate) and the SRH region (auxiliary function in the binding and hydrolysis of ATP) and the C-terminal protease domain. Catalytic Center with Motive HEXXH binding  $Zn^{2+}$  places - according to the MEROPS classification - all AtFtsH in the M4 family.

Tab. 3. Data on genes encoding chloroplast AtFtsH proteases. The table was created from the gene data contained in the NCBI database. Gene length in base pairs

Proteases	Gene	Gene length
AtFtsH1	<i>AtFTSH1</i>	2809
AtFtsH2	<i>AtFTSH2</i>	2445
AtFtsH5	<i>AtFTSH5</i>	3982
AtFtsH6	<i>AtFTSH6</i>	2445
AtFtsH7	<i>AtFTSH7</i>	3982
AtFtsH8	<i>AtFTSH8</i>	2954
AtFtsH9	<i>AtFTSH9</i>	3970
AtFtsH11	<i>AtFTSH11</i>	5266
AtFtsH12	<i>AtFTSH12</i>	5792

### Lon proteases

The proteolytically active form of AtLon4 is a protein associated with strong interactions with the thylakoid membrane on the stroma side [35], this protein is also assigned a mitochondrial location. In the primary structure of AtLon4, we find the AAA + domain which contains the A and B motifs and the C-terminal protease domain. The catalytic center with the SK motif places – according to the MEROPS classification - AtLon4 in the S16 family.

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## PHYSICAL ACTIVITY OF STUDENTS DURING THE COVID 19 PANDEMIC

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### Abstract

Physical activity is an integral part of a healthy lifestyle. It is a broadly understood concept, it is especially related to physical effort, as well as to various physical activities performed voluntarily of their own free will. The article focuses on presenting and characterizing physical activity undertaken by students mainly of the University of Rzeszów in the field of Physical Education and Tourism and Recreation, as well as on determining the level of physical activity during the prevailing Covid 19 pandemic. Taking into account the prevailing situation related to the pandemic, any physical activity is clearly limited, but looking at the results obtained, it can be said that the level of physical activity of students is relatively high. Taking into account the diversity of origins of respondents, their attitude to undertaking physical activity and their achieved general level of physical activity, this allowed to draw appropriate conclusions.

### Keywords:

*health; physical activity; Covid-19*

### Introduction

Physical activity is an integral part of a healthy lifestyle. The purpose of physical activity is primarily to improve one's own well-being, but also to prevent the occurrence of many diseases. Regularity in undertaking physical activity has a positive effect on our body and on the proper functioning of our mind. The aim of the article is to analyze and determine the level of physical activity of students during the Covid 19 pandemic. The research was conducted over a period of two months from January 2021 to February 2021. The research covered 150 respondents studying at the University of Rzeszów, coming mainly from the Podkarpackie Voivodeship. The majority of respondents were women (62%), while men were 38% of all respondents. The diagnostic survey method was used in the research. As a research tool, the International Physical Activity Questionnaire IPAQ was used, which was made available to a group of students of the University of Rzeszów and sent personally via a link to the "survio" survey page. The studied group of students was in the age range of 19 – 25. The largest group of students achieved the highest values in intensive activity. Men show higher MET values than women in all types of physical activity. The largest number of



respondents – 61.6% – achieved a high level. Among the people surveyed, a large number of students are characterized by a normal body mass index – BMI. The level of physical activity of the surveyed students during the Covid 19 pandemic is high. This is evidenced by the relatively high values achieved at a high level. The predominant physical activity among both sexes was found to be intense activity. The level of physical activity of the respondents is influenced by variables such as gender, age, place of residence and body mass index BMI. The study group of students meets the average MET – min / week reaching 7679.53 MET.

## **The concept and meanings of health and physical activity**

Health is an inseparable element of human life. It is not only the absence of disease, but also a state that performs physical, mental and social functions, striving for well-being. B. Woynarowska shows that there are over 300 definitions of health, but none of them uses a sufficiently extensive issue that lies behind the short concept of health [1].

J. Domaradzki, based on philosophical foundations, shows the division of the definition of health into "naturalistic and normative. The former capture health as part of the natural processes taking place in the body. On the other hand, normative definitions assume that the value judgments contained in it are an inseparable element of health, so that it cannot be a neutral term, but is always contextual in nature" [2]. In order to fully understand the definition of "health", different types of health should be distinguished: physical health, mental health (it is divided into emotional and mental health), social health and spiritual health [3]. Physical activity has played a key role in human life for centuries. It aims to improve and maintain proper physical condition. A significant element is resistance to fatigue and stress, as well as improvement of well-being, mood, which positively affect human health. Regular physical activity has a positive effect on the human musculoskeletal, respiratory, nervous and circulatory systems [4]. There are three types of physical activity, the combination of which is a complete physical activity. General physical activity, which is characterized by various types of activities, involving muscles, these include: walking, going shopping, etc. Endurance activity – these are aerobic and endurance exercises that consist in performing dynamic and rhythmic movements, involving large muscle parts. Exercise developing muscle strength and flexibility – we are talking about resistance training, strength training and stretching exercises [5]. A healthy lifestyle is primarily characterized by a larger scale of behaviors that promote health, while avoiding engaging in negative behaviors that do not use our health and related well-being. One of the many determinants that fully affect our body, the condition is physical activity. Taken regularly, it stimulates our whole body to function properly, as well as development [6]. Physical activity reduces stress, improves mood, thanks to the hormones of happiness produced by our body, in other words endorphins, which are released after finished exercise. Endorphins are characterized by various actions that are not only a moment of euphoria, a dose of positive energy and a sense of power, but also have a background with anti-anxiety, analgesic and fatigue effects. The main task of happiness hormones is to calm emotions, negative feelings, reduce the feeling of pain, as well as reduce the shortness of breath favored during sports and to some extent eliminate the feeling of fatigue [5]. It is said that regular physical activity is good for everyone, but there are some health risks for people with chronic or comorbidities. In such cases, before undertaking any physical activity, we should consult a specialist who will select the

appropriate forms of activity. An important problem is a sedentary lifestyle, which has many negative effects, not only problems with the spine, but also serious health problems associated with the lack of any physical activity. When paying attention to a sedentary lifestyle, it should be remembered that the sudden start of intense and prolonged exercise can lead to a limitation of the body's immune response [7, 8].

## **Physical activity during the Covid 19 pandemic**

The coronavirus disease Covid-19 was first detected in Wuhan, China and has spread around the world. Covid-19 is the fifth pandemic after the 1918 flu pandemic [9]. The consequence of the disease is damage to the structures of the alveoli and the accumulation of inflammatory fluid in them, which can lead to respiratory failure [10]. The recommendations to isolate yourself from other people and stay at home are aimed in particular at reducing the risk of contracting Covid-19 and the spread of the virus. In this case, limiting physical activity in the open air and the use of sports facilities is associated with a decrease in the physical activity of people, which can lead to the development of many diseases [11]. Regular exercise along with healthy eating, rest and sleep can improve the physical performance of the body, strengthen its immune defenses and prevent the occurrence of various diseases that can adversely affect health in combination with coronavirus. Therefore, active spending of free time during the pandemic even at home will positively affect the human body and increase the chances in the fight against the virus. During the pandemic, a beneficial effort will be endurance effort, which results in a reduction in the rate of heart contractions and blood pressure at rest [12].

## **Purpose of the study**

The aim of the research is to analyze and determine the level of physical activity during the Covid 19 pandemic based on student feedback. The basic research questions included in the questionnaire focus on the daily physical activity of the students.

The following research questions are presented below:

1. What is the overall level of physical activity of students?
2. Is there a difference between the physical activity of men and women, paying attention to its intensity, frequency and time?
3. What impact do variables such as gender, age, place of residence and body mass index BMI have on the level of physical activity among the surveyed students?
4. Does the surveyed group of respondents meet the MET average?

## **Test material and methods, characteristics of the test group and characteristics of the test site**

The research used the subjective method. As a research tool that will allow to collect relevant data, the International Physical Activity Questionnaire IPAQ, made available to students who have expressed their willingness to take part in the research, was used. Before starting the survey, each respondent received a short information about the purpose of the research and the results obtained,



which will be used only to write a scientific article. Joining the survey required the consent of the study participants. The study used a short version of IPAQ consisting of 7 questions about physical activity performed during the day related to work, everyday life and leisure. The information contains concerns the time spent sitting, walking and practicing intense or moderate physical activity. In addition, 2 open-ended questions were used, in which body height and body weight should be given, 2 closed questions of one choice regarding the sex of the respondents and the place of residence of the respondents, and 1 question regarding the age of the respondents. In the questionnaire, attention is focused only on activities lasting not less than 10 minutes without interruption. The survey questionnaire is anonymous and voluntary and has no time limit. The research used the subjective method and the International Physical Activity Questionnaire "IPAQ" conducted remotely due to the prevailing Covid-19 epidemic, which prevents direct contact with the study participants. The research was conducted over a period of two months from January 2021 to February 2021. The survey was visited by 241 people, of which 95 respondents did not finish answering. The remaining 151 participants of the survey ended the survey positively. 1 survey was rejected due to the lack of height and weight, which is necessary in the calculation of BMI. Each of the surveyed persons received information regarding the completion of the questionnaire and its anonymity. In the end, 150 students took part in the study, including 97 women and 53 men. These were people in the range of 19 - 25. The studied group of people came mainly from the Podkarpackie Voivodeship. These were people studying at the University of Rzeszów. Most of the respondents were students from the following faculties: Physical Education, Tourism and Recreation, Journalism, Pedagogy and Finance and Accounting. The people taking part in the survey were selected on the basis of their status as a student. In the group of respondents, the vast majority are people in the age range of 21-22, while the smallest number of people are people in the range under 19 years. As the place of residence of 84 respondents indicated the city, while rural areas are inhabited by 66 respondents. The research was conducted in electronic form using the online survey system "survio". The condition for joining the research was active study at the University of Rzeszów in Rzeszów. The link to the questionnaire of the survey was made available individually on the social platform "Facebook" and through other people willing to help, thanks to the distribution on their university groups. The survey was posted in groups of students on social networks. A larger number of respondents were students from the Podkarpackie Voivodeship. The results of the research were presented using charts, tables and detailed descriptions.

## **Research results**

On the basis of the declared height and body weight, the average body height was calculated by gender (Tab. 1.) The average body height was calculated by summing up the height of each of the subjects and dividing by their number.

Tab. 1. Characteristics of the parameters of the somatic structure taking into account gender

Parameters	Women		Men		Total average	
	Average	SD	Average	SD	Average	SD
Height (cm)	166	6.04	182	7.67	174	6.85
Weight (kg)	58.86	10.57	83.35	12.89	71.10	11.73
BMI (kg/m <sup>2</sup> )	21.53	3.42	25.06	2.91	23.3	3.16
Min. rise	154		164		159	
Max. rise	180		200		190	
Min. Weight	41		64.5		52.7	
Max. Weight	90		120		105	

Source: based on own research

On the basis of the collected research results, the average body mass index – BMI (Body Mass Index), the minimum and maximum body mass index divided by gender was calculated. The body mass index was calculated by summing up the BMI of each of the subjects studied and dividing by the number of people. The results are shown in Fig. 1.

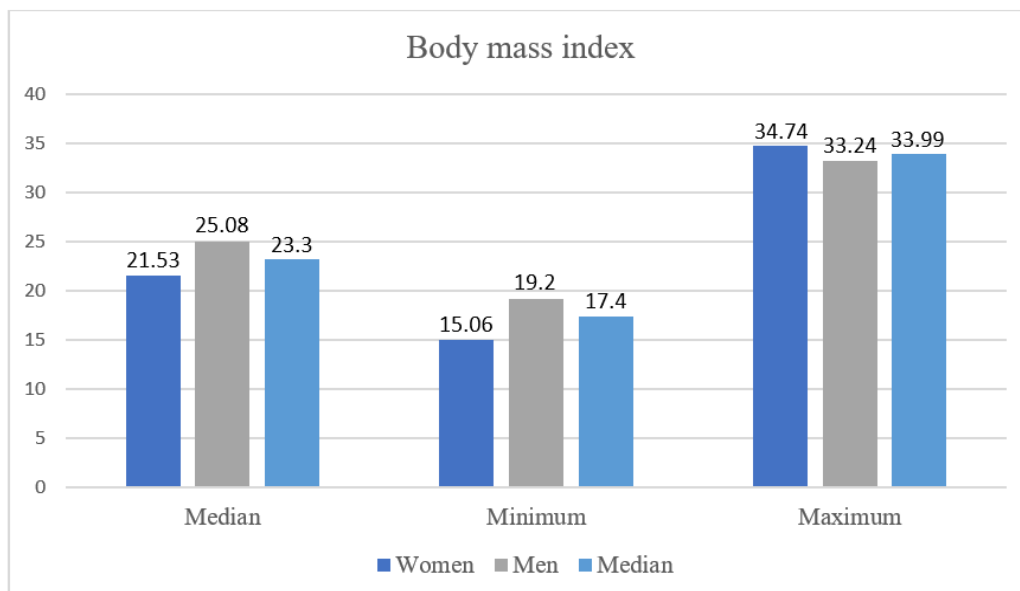


Fig. 1. Average, minimum and maximum body mass index (BMI) broken down by gender

Source: based on own research

Fig. 2. shows the division of women and men according to obesity, overweight, normal weight and underweight. The body mass index – BMI of each of the examined subjects was calculated based on the information contained in the survey, regarding height and body weight. Each respondent was assigned a properly calculated body mass index. Obesity of the first degree is in the range of 30.0 – 34.99, obesity of the second degree – 35.0 – 39.99 and obesity of the third degree above 40, which is considered a disease and the level of threat to life.

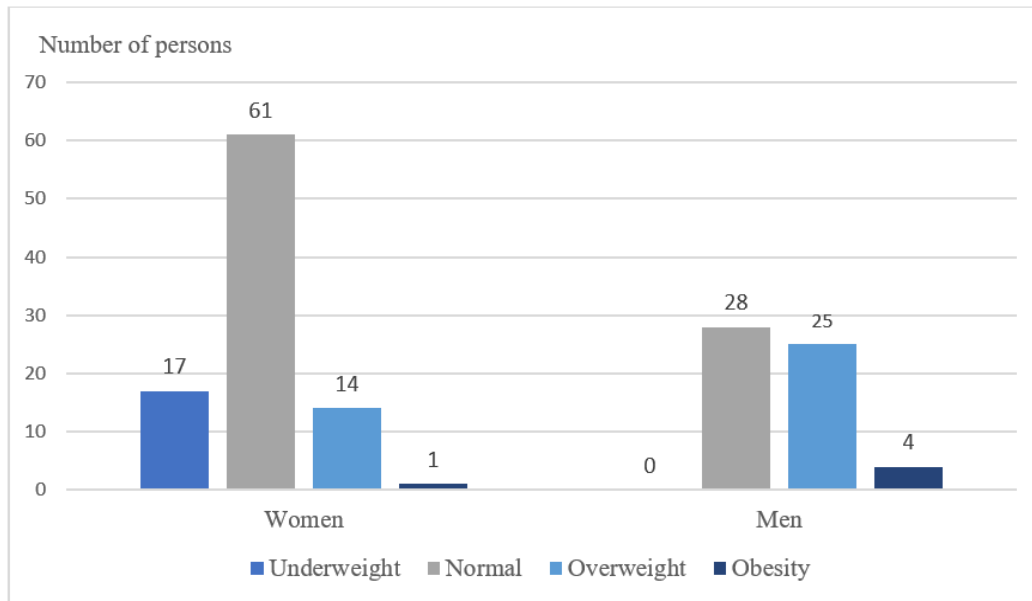


Fig. 2. Division of the subjects according to the values of the body mass index BMI taking into account gender  
Source: based on own research

Fig. 3. shows the breakdown of physical activity by gender. Physical activity is divided into four categories: intense activity, moderate activity, walking, and total activity. The results are presented in MET units (min/week). The given results were calculated by multiplying the coefficient assigned to physical activity by the number of days of its performance per week and the duration in minutes per day. Total activity was calculated on the basis of the sum of the results of each activity.

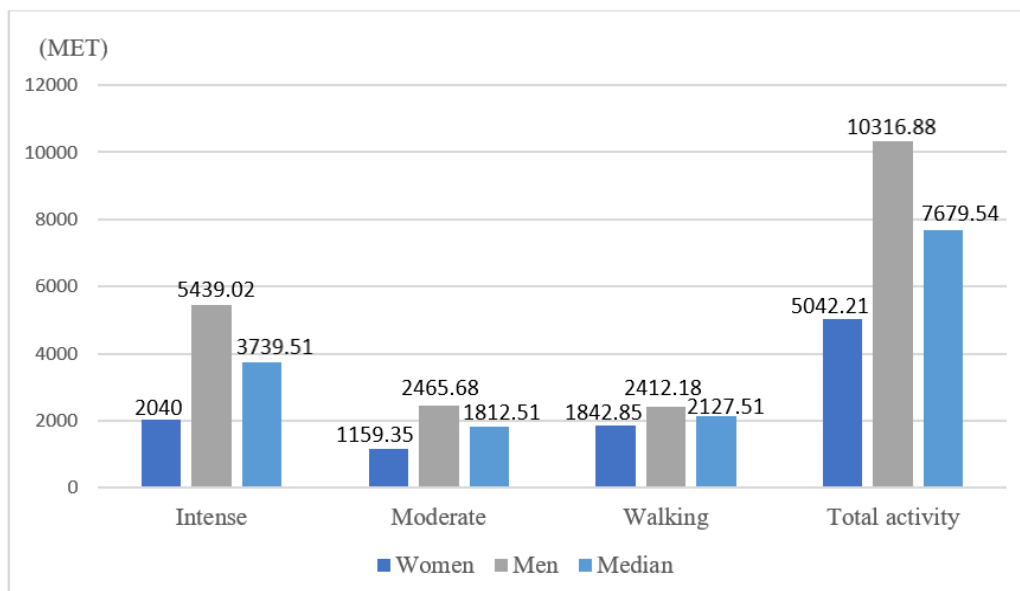


Fig. 3. Division of physical activity by gender  
Source: based on own research

Tab. 2. shows the types of physical activity broken down by gender, mean, median, minimum, maximum and standard deviation. From the results obtained, we can notice a relatively large difference between the physical activity of women and men.

Tab. 2. Types of physical activity taking into account gender

Type of physical activity (MET – min/week)	Gender	Average	Median	Minimum	Maksimum	Standard deviation
Intense	Women	2040.0	960.0	80.0	16800.0	3028.54
	Men	5439.02	2880.0	80.0	19200.0	5975.69
	Average	3739.51	1920.0	80.0	18000.0	4502.11
Moderate	Women	1159.35	480.0	40.0	10080.0	1712.87
	Men	2465.68	1200.0	40.0	13440.0	2946.93
	Average	1812.51	840.0	40.0	11760.0	2329.90
Walking	Women	1842.85	792.0	33.0	13860.0	2479.99
	Men	2412.18	990.0	33.0	16632.0	3384.60
	Average	2127.51	891.0	33.0	15246.0	2932.29
Total activity	Women	5042.20	2232.0	153.0	40740.0	7221.40
	Men	10316.88	5070.0	153.0	49272.0	12307.22
	Average	7679.53	3651.0	153.0	45006.0	9764.30

Source: based on own research

## Discussion and conclusions

The aim of the study was to analyze and determine the level of physical activity of students during the Covid 19 pandemic. The assumed goal has been achieved. Thanks to reliable answers of respondents participating in the survey, answers to previously posed research questions were obtained. The first research question referred to the level of physical activity, wanting to get answers to the question: "What is the general level of physical activity of students?". After a thorough description and calculation of the received answers contained in the survey, it can be concluded that the level of physical activity of the surveyed students during the Covid 19 pandemic is high. The second research question was as follows: "Is there a difference between the physical activity of men and women, paying attention to its intensity, frequency and time?" A significant difference was noted in the undertaking of physical activity by both sexes. Men achieved relatively higher MET values – min/week in all types of physical activity: intense, moderate, total and walking than women. The predominant physical activity among both sexes was found to be intense activity. A significant difference may be related to the more frequent physical activity of males due to more intensive work than women. Another research question was: "What impact do variables such as gender, age, place of residence and BMI have on the level of physical activity among the students surveyed?". Definitely, the level of physical activity of the respondents surveyed is influenced by the above-mentioned variables. Activity with each age is significantly reduced, adolescents are more likely to undertake physical exertion than the elderly. The place of residence also plays a big role, in rural areas physical activity is significantly limited due to the lack of gyms, swimming pools m.in recreational areas. The surveyed group of respondents with a normal body mass index BMI achieves much better results in physical activity than overweight people, which is largely related to health and proper functioning of the body. The last research question refers to the MET-min/week ratio and reads as follows: "Does the surveyed group of respondents meet the MET average?". Analyzing the obtained results, it was found that the studied group of students meets the average MET – min/week reaching high MET values. The mean total activity of both sexes is 7679.53 MET – min/week. The achieved high level of physical activity of students testifies not only to regular physical exertion,

but also to taking care of a healthy lifestyle, which is especially important during the prevailing Covid 19 pandemic. However, the restrictions related to the pandemic did not affect the high level of physical activity of the surveyed students. The hypothesis put forward - "The overall level of physical activity of the studied students is relatively high." is correct and agrees with the obtained research results. The received and thoroughly analyzed responses of the surveyed group of respondents contributed to the confirmation of the previously assumed hypothesis. Research shows that a group of students achieves definitely high MET values by undertaking regular physical activity.

The research conducted by G. Badicu includes students of the Transilvania University in Brasov, 225 men and 139 women. The author presents relatively similar research results to the other authors. Namely, the highest values of physical activity were obtained by men at a high level – 51.76%, a moderate level was achieved by 30.58% of men, and a low level was obtained by only 17.65%. In turn, the highest percentage of women reached the moderate level – 43.16%, high – 33.81%, and the low level was obtained by 23.02% of all women. There is a significant difference among both sexes showing higher physical activity of men at a high level. On the other hand, women achieved higher values than men at moderate and low levels [13].

Foreign research conducted in the United States by Pathare and others. present an important similarity with other authors. In other words, the largest number of students – 48% showed a high level, slightly less 30 respondents indicated moderate activity – 40%. The remaining part of students – 12% declared a low level of physical activity [14].

Foreign research conducted by Mulahasanović, which included students of the University of Tuzli, presents quite similar results to other authors. Namely, the average level of physical activity of men was 6013.493 MET – min/week, while women showed a much smaller average of 4619.381 MET – min/week. Thanks to the obtained results of the study, it was found that the level of physical activity is sufficient. However, subsequent studies show higher physical activity of men than women [15].

Significant differences related to the level of physical activity of students are shown by J. Kościuczuk and others. Most students showed a moderate level of physical activity (67%), 28% of students reached a high level, while only 5% of respondents achieved an insufficient level [17].

In our own research, we can notice a relatively high difference. Students showed a high level of 61.6%. Men showed significantly higher average scores than women at high levels. Women, on the other hand, achieved relatively higher scores than men at a sufficient and low level. The physical activity undertaken by women and men is relatively diverse.

J. Kościuczuk and others in the conducted studies, they showed average values of physical activity divided into intense, moderate, total and walking activity. The overall mean of the respondents surveyed was 3014.5 MET – min/week  $\pm$  1564.8. The highest values of average physical activity were achieved by moderate activity 1158.3 MET – min/week and walking – 1160.6 MET – min/week. In contrast, intensive activity among students was relatively low at just 695.7 MET [16].

Relatively different differences are presented by B. Bergier and others. illustrating different types of physical activity. Total physical activity among the students studied was 2359.5 MET – min/week. Walking – 959.2 MET – min/week and intensive activity – 901.5 MET min/week were indicated as the dominant activity. On the other hand, the lowest values occurred in moderate activity – only 533.6

MET – min/week. Men showed greater values than women in making intense and moderate efforts. Women, on the other hand, achieved higher values in terms of walking [18].

Current research shows significantly higher values of physical activity of the studied students than in other authors. Comparing my own research with other authors, it is confirmed that men achieve much higher values of physical activity than women. The total activity of men is 10316.88 MET – min/week, while that of women is almost half less 5042.20 MET – min/week. The dominant activity among men is intense activity. The other activities are at a very similar level with minimal difference. When it comes to women, their leading point, as in men, is intense activity.

Research conducted by B. Begier and others show the ranges of time spent sitting in one working day. According to the authors, the students studied on average spending time sitting 329 minutes. Men spend more time sitting (347.1), women a little less (311.6). In the studies, there were no differences in sitting taking into account gender [18].

In J. Kościuczuk's research, the average time spent sitting for students was 1850.3 minutes/day on weekdays. On non-working days, the average time was 609.5 minutes/day. From both values, an average total sitting time during the week of 1937.3 min/week (32.3 h/week) was calculated [16].

Analyzing our own research results, we can notice significant differences in the time spent sitting by men and women. Women spend relatively more time than men sitting. Women achieve the highest values in terms of sitting by spending more than 7 hours in one working day. Men, on the other hand, are distinguished by a small time devoted to sitting. The largest percentage of men declared that they spend 2-3 hours a day sitting.

Comparing the current research to the results of research carried out by other authors, a key similarity is the use of the International Physical Activity Questionnaire "IPAQ" as a research tool, which made it possible not only to assess the level of physical activity, but also allowed to determine the number of days and time spent on intense, moderate, total activity and walking.

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## **LOCAL TRAVEL PLANNING ASPECTS TAKING INTO ACCOUNT THE RISK OF COVID-19 PANDEMIC**

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### **Abstract:**

The aim of the study was to analyze the factors influencing local travel planning during the COVID-19 pandemic. The study uses the diagnostic survey method. The research tool was an original questionnaire which was used to survey 106 respondents. The Statistica 13.0 PL program was applied for statistical calculations. Arithmetic means were determined, and the significance of differences was calculated using the Student's t-test and the Pearson's Chi-square test for the confidence interval  $p < 0.05$ . The obtained results show that the means of transport of choice was a passenger car. During trips, tourists most appreciated the companionship of their partner, family and friends. When planning their trips, women would mainly choose several day trips, with the male respondents preferring one-day trips. Nearly all the study subjects organized local trips independently. More than half of them reported that when traveling locally they were interested in both natural and cultural values.

### **Keywords:**

*local travel; planning, COVID-19 pandemic; tourism; leisure time*

### **Introduction**

The state of the development of tourism globally is largely determined by threats that also affect the functioning of tourist destinations. According to Panasiuk [1], threats are the result of natural, social, political and economic factors. Epidemics and pandemics having a significant impact on the state of tourism are definitely one of such phenomena.

The COVID-19 pandemic undeniably contributed to a serious crisis in the tourism industry and changes in the current travel plans, arousing tourists' interest in the immediate vicinity to a large extent [2]. Beyond a doubt, domestic tourism, and thus also local tourism, has gained increasing importance in recent times [3]. It should be expected that in the near future the current epidemiological situation, both in places of tourist traffic as well as in particular tourist destinations [4], will significantly affect tourists' travel decisions. The COVID-19 pandemic has motivated many researchers to undertake studies on local tourism in the time of the pandemic. According to the literature, local tourism has gained only negligible importance during the COVID-19 pandemic and



still has few supporters [3]. Nevertheless, Zawadka et al. [4] proved that the COVID-19 pandemic significantly changed respondents' travel plans [4]. A large percentage of tourists decided not to travel abroad in favor of a less popular but at the same time safer places in their home country [4]. It is comforting, however, that despite the threat of the pandemic, people do not give up local travel [3].

The aim of the study was to analyze the component factors influencing local travel planning, taking into account the risk of the COVID-19 pandemic.

## **Materials and Methods**

The study used the diagnostic survey method performed by means of a research tool, namely the original questionnaire which contained five closed questions. The five-point Likert scale was applied with respect to the question concerning the most-preferred means of transport as well as the identification of companions during a local trip. The survey was conducted in May 2021 via the Internet. The study group enrolled a total of 106 persons involved in the tourism industry, either professionally or as a hobby. The determinant applied in order to divide the respondents was their gender. A vast majority of the respondents were women (69.8%), with men accounting for 30.2% of the study subjects. The age of the respondents was also an important determinant. The largest group, as much as 77.36%, constituted people under the age of 25. A significant minority among the respondents were people aged between 25 and 65 (18.87%) and those over 66 (3.77%). More than half of the study subjects were economically inactive, i.e. students (75.47%). The study group also embraced blue-collar workers, white-collar workers and the unemployed, accounting for 13.21%, 7.55% and 3.77% respectively. Quite a large group of the respondents were city dwellers - 62.3%, compared to rural residents who constituted only a small proportion - 37.7%.

Statistical analysis of the obtained results was performed with the application of the Statistica 13.0 PL program. Arithmetic means were determined, while the significance of the differences was calculated using the Student's t-test and the Pearson's Chi-square test for the confidence interval  $p < 0.05$ .

## **Results and Discussion**

The data analysis shows that the most frequently chosen means of transport for travel on a local scale, both among women and men, was a passenger car (Tab. 1.). This is probably due to the comfort of travel, independence, freedom, the possibility to change plans at any convenient time, as well as greater safety, which is particularly important in pandemic times. Among the advantages of traveling in a passenger car, Sierpiński also mentions freedom in the choice of the route, the car's load capacity, short time of journey to the destination as well as safety from strangers [5]. The performed analysis of the obtained results shows that bicycle trips were also very popular, however declared by men to a greater extent. Hebel and Wyszomirski noted that the bicycle is increasingly important, especially in urban travels, with the respondents choosing this means of transport for health reasons and for pleasure [6]. In the case of traveling by car and bicycle, a statistically significant difference in the scores was found in respect of the studied variable,  $p=0.0183$  and  $p=0.0004$  respectively. Statistical analysis showed that women rated traveling by car

significantly higher than men. The average of such responses was 4.30 in the case of women, compared to 3.69 among the male respondents. The survey shows that traveling by bike was assessed significantly higher by men, which was statistically proven. The average of such responses was 3.13 in the case of men compared to 2.14 in the case of the female respondents. The least frequently chosen means of transport for local travels was the train, as well as the bus or the minibus. Sierpiński classified the disadvantages of urban public transport as, among others, high congestion, the lack of possibility to decide on the route, the need to adjust one's own time to the timetable, as well as the frequent lack of seats [5]. The obtained results also enable observation that walking trips are of relatively great importance for the modern tourist, especially for women. No statistically significant differences were reported between the study groups for travel by train, bus or minibus, or walking trips.

Tab. 1. The most frequently chosen means of transport for local travel

Means of transport	Gender of respondents		The value of the t-test	p value
	Women	Men		
Car	4.30	3.69	2.40	0.0183*
Train	1.97	2.19	-0.756	0.451
Bus/Minibus	1.89	1.81	0.295	0.768
Bike	2.14	3.13	-3.636	0.0004*
On foot	2.97	2.88	0.336	0.737

\* significant differentiation of variables at the level of  $p < 0.050$

Source: own study based on research material

The conducted research indicates that the choice of a companion is a rather significant criterion influencing local travel planning issues during the COVID-19 pandemic. The data below supports the conclusion that women are most likely to travel locally with a partner, indicating this at a significantly higher rate than men (Tab. 2.). The average of such responses for women was 3.46, and for men 2.25, with  $p=0.0013$ . This proves a statistically significant difference between the indicated averages. Men, on the other hand, are most likely to travel locally with friends. The fact that during the pandemic tourists decide not to travel in an organized group, but to explore the nearest areas in groups of no more than several people is a positive sign. According to the data, supporters of micro trips during the COVID-19 pandemic most often appreciated the company of a partner, family and friends. The selection of companions during a local trip is influenced by the age of tourists and the period of life they are in. As noted by Pytel and Szkup, seniors most often travel with their families (77%), and least often with their friends (8%) [7]. In turn, in the case of adolescents, these preferences are slightly different. It turns out that young people most often explore local areas with their friends (44%), much less frequently with family (29%) and a partner (27%) [7]. What is more, the present study also found that during the COVID-19 pandemic, individual trips also gained importance. Men (2.44) were interested in this form of travel much more often than women (2.08). According to Lubowiecki-Vikuk, among the perspectives of Polish tourism development, there is a tendency for increased activity of single tourists who will travel individually with an orientation towards effort and physical activity [8]. The data presented in the table below also show that the respondents are reluctant to travel in the area with completely random, unknown people.

This type of companionship was preferred by the male respondents (1.19) rather than the female subjects (1.11), however, these differences were not significant.

Tab. 2. Choosing companionship to travel in the immediate area

Companionship	Gender of respondents		T-test value	p value
	Women	Men		
With a partner	3.46	2.25	3.298	0.0013*
With family	3.14	3.40	-0.866	0.389
With friends	3.14	3.63	-1.669	0.098
Alone	2.08	2.44	-1.134	0.259
In an organized group	1.51	1.94	-1.634	0.105
With random people	1.11	1.19	-0.855	0.394

\* significant differentiation of variables at  $p < 0.050$

Source: own study based on research material

The data obtained shows that nearly half of the respondents (45.28%) indicated that during the COVID-19 pandemic, tourist mostly preferred trips lasting several hours, which was declared to a greater extent by women than men (Tab. 3.). One-day trips were slightly less popular (39.62%). The data analysis demonstrates that women most often chose several-day trips, with men preferring one-day trips. The least popular type of travel were weekend trips, accounting for 15.09%. Undoubtedly, the duration of a trip depends on the choice of the means of transport. Cieszewska et al. [9] in their analyzes confirmed that amateur cycling enthusiasts with or without the company of children most often choose trips lasting several hours or all-day trips, while lovers of organized bicycle trips and universal off-road cyclists prefer one-day or weekend trips. The results of the research presented in the paper in question are also confirmed by the data of the Central Statistical Office (GUS) indicating a 65% decrease in the number of tourists using tourist accommodation facilities observed in March 2020, compared to the same period in the previous year [10]. It should be noted that the value of the Pearson's Chi-square test did not show any significant correlation between the variables. This value was 5.396 with  $p=0.673$ . When analyzing the obtained results, it can also be stated that the criterion of the duration of a trip to the immediate area during the COVID-19 pandemic was more important for women than for men.

Tab. 3. Average duration of a trip in the immediate area

Number and % of the total	Duration of a trip	Gender of respondents		
		Women	Men	Total
Number	Several hours	38	10	48
% of the total		35.85%	9.43%	45.28%
Number	One-day	24	18	42
% of the total		22.64%	16.98%	39.62%
Number	Weekend	12	4	16
% of the total		11.32%	3.77%	15.09%
Number	Overall	74	32	106
% of the total		69.81%	30.19%	100%
Person's Chi-square: 5.396, n=0.673				

\* - Person's Chi-square value shows significant correlations at  $p < 0.050$

Source: own study based on research material

The results of the conducted research indicate that during the COVID-19 pandemic, almost all the respondents participated in self-organized trips, and this percentage was as high as 96.23% (Tab. 4). Women participated in such trips much more frequently (66.04%) than men (30.19%). Trips organized by travel agencies, in which only women declared their participation, reached a very low value of less than 4%. It is undeniable that from an economic point of view, individually organized trips are much cheaper than those organized by travel agencies. Nowadays, financial issues are quite important due to the crisis caused by the pandemic as a result of which a great number of people have lost their regular income. According to the data of the Association of Entrepreneurs and Employers (ZPP), the closure of the economy increased unemployment in EU Member States by 2.7% [11]. In turn, Rożek [12] reports that due to group layoffs during the COVID-19 pandemic, 15,000 employees lost their jobs in Poland and the effects of the crisis on the Polish labor market affected men more than women. It is therefore understandable that Poles are careful with money when organizing trips. Widomski points out that the proximity of destination and low costs are the main determinants of choosing a holiday in Poland [13]. The analysis of the data contained in the table below shows that the respondents did not participate in any trip organized by their employers or other institutions. However, one positive aspect is that due to the constantly growing number of confirmed cases of COVID-19 infection, employers resign from organizing such trips out of concern for the health and safety of their employees. The value of Pearson's Chi-square test did not indicate any significant correlations between the variables.

Tab. 4. The method of organizing a trip on a local scale

Number and % of the total	The way of organizing the trip	Gender of respondents		
		Women	Men	Total
Number	Trip organized by a travel agency	4	0	4
% of the total		3.77%	0.00%	3.77%
Number	Trip organized by the workplace or other institution	0	0	0
% of the total		0	0	0
Number	Trip organized independently	70	32	102
% of the total		66.04%	30.19%	96.23%
Number	Overall	74	32	106
% of the total		69.81%	30.19%	100%
Person's Chi-square: 1.797, p=0.180				

\* - Person's Chi-square value shows significant relationships at  $p < 0.050$

Source: own study based on research material

The analysis of the obtained research results (Tab. 5.) demonstrated that the selection of tourist attractions, which play a unique role when visiting the immediate area, is an important criterion when planning local trips during the COVID-19 pandemic. More than half of the respondents (66.04%) declared that they are interested in both natural and cultural values (Tab. 5.). Whereas nearly 30% of the study subjects confirmed their interest in natural values solely and exclusively, and this answer was indicated much more often by women than men. This may result from the introduction of the state of epidemic threat in 2020 [11], followed by lockdown, which in consequence arouse tourists' desire to stay close to nature and systematically oxygenate the body. The remaining percentage of the respondents (5.66%) are supporters of only cultural values. When planning a trip

on a local scale, cultural values were most often chosen by men, with the value of 3.77% compared to only 1.89% among the female respondents. According to the data of the Statistical Office in Warsaw concerning museums in the Mazowieckie voivodship during the COVID-19 pandemic, a 47% decrease in the number of visitors was observed in the whole year 2020 [14]. The popularity of national temporary exhibitions, educational events in museums and museum collections decreased by 35.6%, 62.1%, and almost 16% respectively [14]. On the other hand, research conducted by Kruczek shows that among the ten most frequently visited attractions in Poland (available after purchasing a ticket), the Tatra National Park, The Palace in Wilanów and the Palace in Łazienki are in the lead [15]. The data contained in the table below allow for the conclusion that the value of the Pearson's Chi-square test did not indicate any significant correlations between the variables.

Tab. 5. Tourist values playing a unique role when visiting the immediate area

Number and % of the total	Values	Gender of respondents		
		Women	Men	Total
Number	Natural values	22	8	30
% of the total		20.75%	7.55%	28.30%
Number	Cultural values	2	4	6
% of the total		1.89%	3.77%	5.66%
Number	Everything interests me	50	20	70
% of the total		47.17%	18.87%	66.04%
Number	Overall	74	32	106
% of the total		69.81%	30.19%	100%
Person's Chi-square: 4.05, p=0.132				

\* - Person's Chi-square value shows significant relationships at  $p < 0.050$   
Source: own study based on research material

## Conclusions

The conducted analyzes lead to the conclusion that all of the factors subject to assessment in the present study have a significant impact on the issues of planning a trip on a local scale, taking into account the risk of the COVID-19 pandemic. The most frequently chosen means of transport for local journeys during the COVID-19 pandemic, both among the male and female respondents, was the passenger car. Cycling, declared to a greater extent by men, was also very popular. On a local scale, women most often travel with a partner, while men with friends. It was also noted that during the COVID-19 pandemic, individual travel gained importance as well. Women most willingly chose several-day trips, compared to men preferring one-day trips. Weekend trips were the least popular among the respondents. Almost all the study subjects took part in journeys organized independently. Very low value was reached by trips organized by a travel agency, in which participation was declared only by women. More than half of the respondents stated that when traveling in the immediate vicinity, they are most interested in both natural and cultural values. Approximately 30% of the study subjects declared their interest in natural values solely. The remaining percentage of the respondents are supporters of cultural values exclusively.

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## **EU MIGRATION POLICIES AND SECURITIZATION OF MIGRATION**

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### **Abstract:**

The Article focuses on the background of the migration, the securitization of migration and how it has impacted the EU as a whole. Firstly, the Article is going to emphasize on the dynamics of securitization of Migration and how it has effected the EU and its policies since 1990. In the same topic few examples which is help to understand the fear and the link between migration, terrorism, and security will be discussed. Then Article would focus on explaining the migration within the framework of security which includes, but not limited to, military, economic, environmental, social and political security. The explanation and the relationship between these different securities is based on the concept presented by Copenhagen School. Further in the same topic the three distinct units of Securitization will also be elaborated. Last but not the least the three main issues of securitization of migration namely Internal security, identity and welfare state will be briefly explained.

### **Keywords:**

*EU migration policy; security, securitization; Copenhagen School*

### **Introduction**

After the Arab Spring, migration and educational issues related to migration have started being considered in a new dimension. The issue of migration has gained a crucial place in both international politics and EU national security policies. Given the example of EU, it has moved to a very important point 9/11 terrorist attacks, and has also influenced EU security policies in migration context; consequently, migration policies have been moved from "low politics" to "high politics" in the EU agenda.

It is apparent that a security-oriented approach is dominant while agreeing on a common migration policy in the EU. As a matter of fact, while common policies were developed on the fight against irregular migration and border controls, issues related to legal migration were not transferred to the supranational area. Thus, in this studies, EU migration policy will be analyzed under the light of securitization theory. International migration has become one of the issues added to the security agenda of states and among the threat perceptions, as well as areas such as environmental problems, energy, and organized crime just after the end of the Cold War.

In order to examine this threat structuring, securitization theory was developed by the Copenhagen Security School, and this theory is applied to various fields including migration. This article aims to evaluate the development of the EU common migration policy within the context of securitization.

The securitization of migration in Europe takes place on the basis of three issues which are Internal security, identity and welfare state. This research reveals that cultural and internal security concerns play an important role in the securitization of migration at the EU level. However, it is argued in this study that Europe, which requisites to become an important economic, political and security power in the integration process, mainly securitizes migration on an economic basis.

## **Global dynamics of Securitization of Migration and its effects on the EU**

International migration has risen to the top of the international security agenda in recent years. Politicians in the US, Europe, and other countries around the globe increasingly link immigration policies and national security. The majority of these discussions concentrates on the relationship of migration flows with international terrorism. Before the events of September 11, the relationship between globalization, migration and security took place in policies and security studies. The bombings that took place in Madrid on 11 March 2004 and in London on 7 July 2005 reinforced existing fears in Europe about the link between migration and terrorism. The bombing of the Paris metro system by an Algerian armed group in 1995 and the attacks of the Kurdistan workers' party in various western European countries in the 1990s have raised concerns about the relationship between migration and security.

Migration and immigrants seem to be closely linked to national security concerns throughout many years. States have traditionally formed their national migration policies in line with their security and economic interests. In this context, the management of international migration flows is one of the areas where politicians make cost-benefit analysis within the scope of certain policies. As such, the effects of migration on international security and other policy areas such as social welfare and economic growth are taken into account while policy-making in this area.

Looking at the framework of immigration control issues before and after September 11, some differences emerged in the EU as well as in the USA. The context of migration security has been at the center of the EU agenda since the 1990s. As a result of the strengthening of far-right parties in many European countries in the 1990s, anti-immigrant discourses spread to all EU countries in a short time and immigration controls were tightened in the light of these discourses. With the events of September 11, open ties have been established between migration, terrorism, security and borders in the EU, and this has led to security acts. Three examples of this effect can be given in the period after the events of September 11, 2001. On 20 September 2001 the European Council on justice and home affairs held an extraordinary meeting, calling for the Commission to urgently examine the relationship between the maintenance of internal security and compliance with international protection obligations and instruments [2]. This first response to the events of September 11 brought immigration to the agenda and associated it with security. A tension has been constructed here between the need to ensure security and the obligation to comply with international legal obligations on asylum and migration. This situation reveals the assumption that human rights and asylum regimes are abused by actual and



potential terrorists. This initiated the act of securitization. As a result of this call, the Commission published a document on the development of a common policy on combating illegal immigration on 15 November 2001. The document emphasized the need to strengthen border controls to combat them by linking them with "terrorist risks" and "crime networks". There is the assumption that insecurity will come from outside and that immigrants are a major source of insecurity for the EU [11]. Following the EU institutions' response to these events, these emphases became more concrete at the Laeken Summit held on 14-15 December 2001, as follows. Although the urgency eased after this extraordinary process, securitization in immigration policies continued. However, there is a differentiation in revealing the framework of the migration control issue in Europe. One of the reasons for this is the problem of observation, hence, irregular entry, stay and employment are difficult to observe and measure. Therefore, there is a high degree of epistemic uncertainty in this policy area. As a matter of fact, evaluation of the scale and significance of this problem is open to different arguments.

The second feature of this field indicates it as exposed to a populist approach. Governments often attempts to show that they control irregular migration, but businesses are also vulnerable to pressures against these practices, such as civil liberties concern and judicial restraints. These concerns make it impossible for any liberal democratic state to achieve the immigration control demanded by populist politics and the media. The issue of irregular migration is an important target for the opposition and the media regarding the loss of state control. The third element is human trafficking in unsafe methods. These networks fall under the jurisdiction of the police as they are closely related to international criminal structures [16]; thus, the discourse of securitization is observed in Europe in this regard as well. The common feature of all three elements is that the preferred solution is "exclusion". Whether the issue is irregular migration, welfare state abusers or human smugglers, the recommended solution is to block entry or deport unwanted migrants. The discourse and policy debates observed at national and EU level focus on various policy measures aimed at the exclusion of migrants: These are; restrict entry into the country through restrictive visa policies; carrier liability and border controls; reducing overstaying through detention and deportation; imposing various penalties to deter irregular migration, labor and abuse of asylum systems.

## **Explaining Migration in a Security Framework: Copenhagen School**

One of the most crucial novelties brought by the Copenhagen School to security studies is that it rejects to approach security only from the military security point of view, but includes economic, environmental, social and political security to the field of security. The purpose here is to divulge that military threats are not the only source of insecurity for societies and people in particular. As an important benefit of examining security with this understanding, it explains the sorts of special relations between these areas and exposes the interaction between these areas. When considering relations shaped by these sectors, the military sector is associated with relations of pressure, and the political sector with relations such as authority and management status [14]. Looking at the economic sector, we perceive that it is associated with various economic arguments such as trade and production [12]. Identity building comes to the fore in the social sector. Finally, in the environmental sector, human activities and the world's biosphere relations stand out.

As an area where this commitment is experienced, security comes to the forefront. However, since threats to states and feelings of insecurity are more related to the degree of closeness, the regional security argument becomes important, and has established its place in the work of the Copenhagen School.

According to the Copenhagen School, securitization has three distinct units: reference objects, securitizing actors, and functional actors. Reference objects are the argument that is perceived under threat. The act of securitization is only successful if the audiences concerned are convinced that there is a threat to the existence of the reference object. In general terms, the traditional reference object of securitization is the state. However, securitizing actors can hold of almost anything as an object of reference, such as national identity, social groups. A securitizing actor is an actor who makes a security discourse about an issue with the aim of securitizing it such as Political elites, army, government, pressure groups. Functional actors, on the other hand, those actors significantly influence the decisions to be taken in the field of security. Overall, Governments and elites are in an advantageous position compared to other actors in influencing society. Every process of securitization by actors includes political and security-related actions, and the act of securitization fails or succeeds, provisional on the persuasiveness of the discourse [15].

Wæver, Buzan, and Wilde define securitization as the successful construction of a problem as an "existential threat" [9]. The securitization approach has presented a different perspective by positioning and defining security as a discursive and political power beyond being an objective situation. Lately, immigrants are the most instrumentalized people in Europe lately. The claim that immigrants harm the state and social identity which is increasingly being articulated by far-right and nationalist parties. As it can be understood from the Copenhagen School's explanation of migration in a security framework, the EU's migration regime tends to integrate migration into a security framework.

It can be held that during the 2015 refugee crisis, migration in Germany was not existentially structured by any actor towards an object of reference (national identity, social groups, individuals, etc.). In this context, the actors of de-securitization were Angela Merkel and the German government. German Chancellor Angela Merkel has advocated the humanitarian approach to the refugee crisis as it comprises of economic and social aspects which was used to some extent to 'import' a new workforce [7]. In the process of de-securitizing migration in Germany, the functional actors were churches, non-governmental organizations and the media. Such functional actors addressed the message that refugees are in danger and need to be assisted and protected. The facilitating conditions in this process were the economic interests of Germany. Therefore, Germany's economic needs has prevented the securitization of migration. Germany's negative population statistics can be seen as the second facilitating condition in the process of de-securitizing immigration in Germany due to the rapid increase in the elderly population in the country.

## **Internal Security, Identity and Welfare State**

### **Internal Security**

The securitization of migration in the European Union in terms of internal security is limited. In this sense, the European integration method ensures that immigration is seen as a matter of internal

security [13]. In other words, economic integration and the securitization of the internal market, which is tried to be created in this direction, serve as the key dynamics in the securitization of migration. The basic condition assumed is the free movement of international services, capital, goods and persons after the abolition of internal borders. However, the fact that this situation will reveal the effects that disrupt the public order and legal order creates fear.

It is also imperative that the EU implement strengthened and harmonized policies for these raised walls. This obligation finds itself in the Schengen Agreement, which laid the first bricks of the aforementioned walls.

The element of crime and foreigner seems to be integrated in the concept of asylum seeker and immigrant. While the EU desires to protect the freedom and welfare area which has been created against this concept, it wants to raise its external borders from land and sea, and attempts to turn the myth of "fortress Europe" [10] into reality by building stronger and thicker walls with harmonious policies as soon as possible. However, it is possible to follow a migration perspective that changes according to countries from the discourses of the media and political elites.

### **Identity**

The EU integration process presents an interesting dilemma in terms of securitization of identities, just as in the securitization of borders. It both helps the development of the presentation of migration as a cultural danger and struggles by trying to prevent the securitization of the concept. In this context, Huysmans highlighted 3 concepts: the cultural importance of border control and the limitation of free movement, the integration problem of immigrants or their assimilation within European societies, and European integration [6]. First of all, it can be thought that the controls at the borders are not only an internal security problem, but also a cultural problem in the EU. Considering that the majority of illegal immigrants and asylum seekers come from 3rd world countries, cultural and racial differences from European societies can be easily comprehended. In response to the situation created by these factors, racism provides the securitization of cultural identity.

The EU and Member States, which are seeking to create a healthy whole as a modern project, take a stand in favor of creating a balanced and classifying system and handle ethnic and cultural differences that disturb the social order. The most important theme in resolving identity and identity-related situations before they become security problems can be viewed as the integration of immigrants from EU societies into their own societies. This integration policy is a crucial cornerstone in the creation of a multicultural society [8]. Immigrants who have obtained their political rights will be able to exist in a multicultural society by integrating into the society. However, this request is not welcomed by the groups that support nationalist homogenization, but can also be seen as a policy that is reacted to. For example, pro-integration policies are securitized by making the internal stability of the society fragile as they have different lifestyles and cultures, which disrupts the homogenized society with immigration and immigrants [6]. In addition, establishing a migration policy that supports the multicultural society that is being tried to be created in the EU and opposes the rising racist voices is also an important agenda item. The reaction to multiculturalism and racism, on the other hand, is the EU's response to the balance of power and disintegration in the pre-World War II situation, and the current securitization of this fear, as Weaver mentioned, according to Huysmans

[7]. When EU immigration policy is associated with this fear, it is in two opposing practices. On the one hand, European migration policy defines immigrants in social and political terms that are negative in terms of stability, emphasizing that they are at the border of societies and displaying a more exclusionary tendency.

### **Welfare State**

Europe is a migration region and the number of people accepted as foreign citizens in the population of European countries is increasing day by day. The fact that such increase also raised unemployment rates which has been the most talked issue at the point of the crisis of the welfare state. However, some numerical data are not compatible with this discourse, say, while Germany had a foreign population of 7.4 million in 2002 and 2003, the unemployment rate was 7.4% [5]. Despite the foreign population falling to 7.2 million in the last two years, the unemployment rate rose to 9.6% [5]. Although the answer to the question "Is migration and unemployment linked to each other?" is not clear in this context, it is seen as "No". These two concepts come to the fore in terms of benefiting from the opportunities of the welfare state and the structural problems experienced by the economy, and are reflected as the basis of the problem.

The definition of community and society to be made over Europe also reveals the difference between desired and unwanted immigrants [1]. This distinction consists of perceptions for national resources and the welfare state. The word perception is important because the EU immigration policy is based on the word perception and perception supports the discussion of immigration-related issues such as welfare, resources, integration, social cohesion and security [4]. Faist thinks that immigration is used by EU politicians and institutions as a "commodity" issue. He states that economic, social and security problems, such as unemployment, housing problem, and the increase in crime rate, create a dilemma for us and them by drawing the reaction of the society on immigrants [3]. This commodification leads to Europe's use of immigration as an internal method in its welfare state arrangements. It is done by excluding a group from the support of the welfare state, by welcoming a group and rejecting it from the beginning, reaching a stage of regulation.

### **Conclusion**

It can be concluded that the migration has become a meta-issue these days in the EU and has become a very powerful theme via which the different policy problems, for instance: identity control and visa policy, asylum applications, integration of immigrants, distribution of social entitlements, and the management of cultural diversity are connected and traversed. So further measure needs to be implemented by EU in order to better manage the migration which could help in abolishing illegal border crossing, trafficking of human beings, etc. The EU should strengthen its relationship with other non-EU neighboring countries. In addition to strengthening its securitization in Migration, EU shall also educate member states and emphasize on human rights, freedom of movement plus include the new countries which entered EU into Schengen zone. For now, there are several developed EU countries which feels threatened by the migration of the people from developing EU countries as they risk loss of jobs, or they are too rigid on the fact that these countries might not be able to adapt their cultural society. Supporting the construction of destabilizing factors and dangers in policies regulating

the membership of a community which could makes the inclusion of immigrants, asylum-seekers and refugees in the EU more difficult. It also indirectly impacts the solidarity, social integration, cultural identity, civility and public order that is promoted in the community.

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## CLICK CHEMISTRY AS A TOOL FOR THE SYNTHESIS OF NEW CAFFEINE DERIVATIVES

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### Abstract:

Caffeine is an alkaloid that shows a variety of pharmacological activities. It has antibacterial, antifungal and antioxidant properties. Phthalimides are organic compounds with various biological activities. This article shows a synthesis method of new triazole analogs of caffeine with phthalimide moiety.

### Keywords:

*caffeine; phthalimide; triazoles; click chemistry*

### Introduction

Caffeine (1,3,7-trimethylxanthine) (Fig. 1.) is an organic compound from purine alkaloids groups. It is a white crystalline solid, odourless with a bitter taste. This alkaloid occurs in coffee beans, tea leaves, cocoa beans and guarana fruits. Therefore caffeine is also known as theine or guaranine. It is also known as a stimulating substance. Caffeine has a lot of valuable properties: antifungal activity, antibacterial activity, raising blood pressure. It is also an antioxidant.

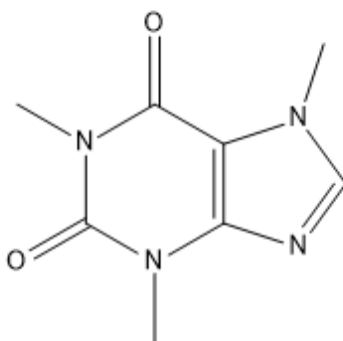


Fig. 1. Structure of caffeine  
 Source: own elaboration

Other compounds with biological activity are phthalimide derivatives (Fig. 2.). Phthalimide is an organic compound from the imides group. Properties of phthalimide are, among other things, anti-inflammatory, anticonvulsants, anticancer and hypoglycemic [1, 2]. The structure of phthalimide

causes easy biodistribution through the body. It is a hydrophobic and inert molecule [3]. Drugs based on phthalimide structure are used in human immunodeficiency virus treatment (HIV) [4] and against autoimmune skin disease [5]. The most popular drug with phthalimide moiety is thalidomide. Thalidomide had been sold to pregnant women since doctors discovered its teratogenic properties.

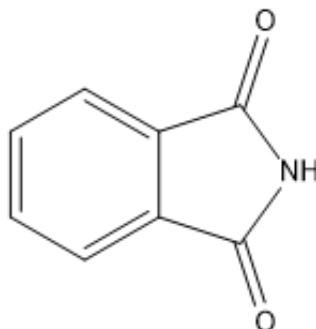


Fig. 2. Structure of phthalimide  
 Source: own elaboration

The main goal of our research was to connect caffeine and phthalimide molecules by triazole ring using click reaction. Triazole is a five-membered ring with three conjugated nitrogen atoms (Fig. 3.). Triazole-based compounds are used as fungicides (Fluconazole, Propiconazole), as plant growth control agents (Paclobutrazole). Triazole compounds block their biosynthesis and affect plant growth enzymes such as gibberic acid or cytokinin. Triazoles also increase antioxidant resistance without harming the plants [6].

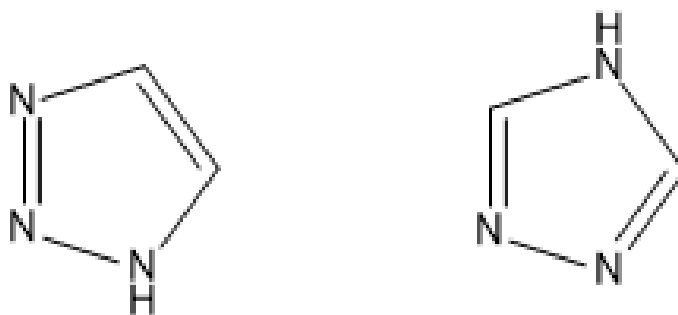


Fig. 3. Structure of 1,2,3-triazole (left) and 1,2,4-triazole (right)  
 Source: own elaboration

Connection caffeine and phthalimide moieties by triazole ring may increase the biological activity of the new compound by inducing a synergistic effect of the linked molecules or eliminating their undesirable side effects.

A click chemistry reaction called 1,3-dipolar cycloaddition, occurring between the azide and the terminal alkyne carbon atom. It was observed at the beginning of the 21st century. Click reactions are highly efficient, they do not require an anhydrous environment, and the products of these reactions show high thermodynamic stability. These reactions can take place under various conditions with the participation of various catalysts. If  $\text{Cu}^+$  is used (Copper Catalyzed Azide-Alkyne Cycloaddition) 1,4-disubstituted 1,2,3-triazole will be formed. When  $\text{Ru}^+$  (Ruthenium-Catalyzed Azide-Alkyne



Cycloaddition) is used 1,5-disubstituted 1,2,3-triazole will be formed; however, to obtain a mixture of isomers, the elevated temperature must be used [7]. The differences between the reaction products are shown below.

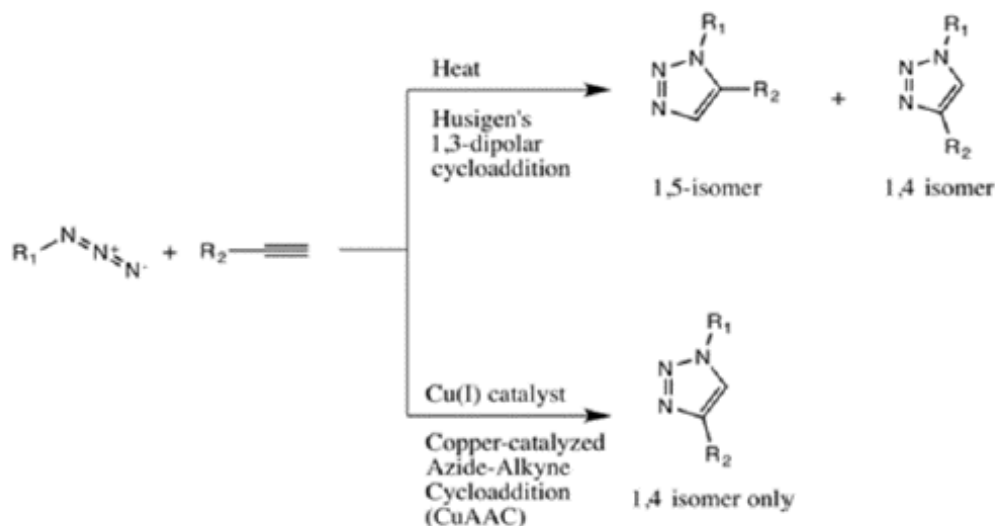


Fig. 4. Differences between reactions  
Source: own elaboration

In the research, syntheses were carried out to obtain a series of derivatives with different number of carbon atoms in the aliphatic chain ( $n = 3,4,5,6,8,10,12$ ). Obtained compounds were analyzed by FT-IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and ESI-MS. The energy of the obtained compounds was calculated using the Gaussian program. Shortly, biological tests will be carried out to determine the activity of the compounds.

## Experimental

### Synthesis of compound 1

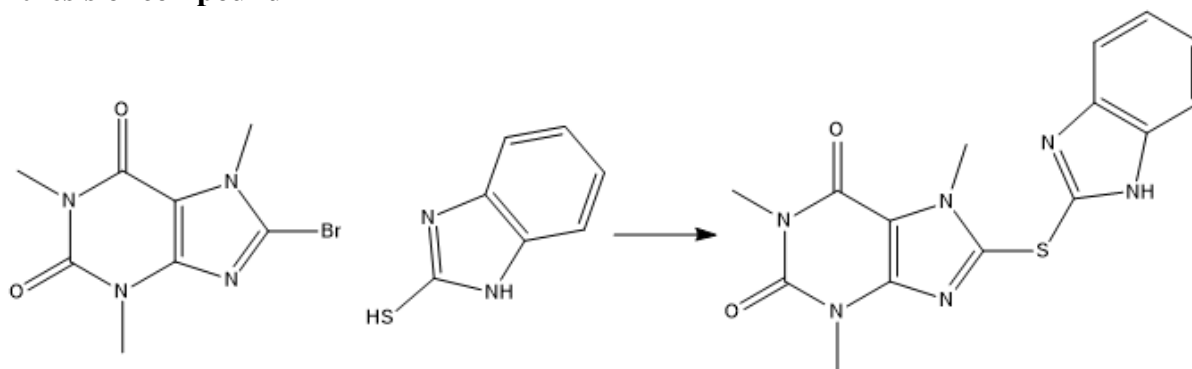


Fig. 5. Scheme of synthesis compound 1  
Source: own elaboration

Benzimidazole (225 mg, 1.5 mmol) was dissolved in 8 mL of EtOH, placed under stirring. NaOH (80 mg, 2 mmol) dissolved in 1 mL  $\text{H}_2\text{O}$  was added. After 30 min, 8-bromocaffeine (273 mg, 1 mmol) was added. Stirring was terminated, and the refluxing of the solvent commenced. After about 16 h of

heating, the mixture was cooled to room temperature and then poured onto ice. It was allowed to evaporate. The obtained product was crystallized from methanol. A crystalline white solid was obtained. The progress of the reaction was monitored by TLC plates (petroleum ether: ethyl acetate, 3:7).

$C_{15}H_{14}N_6O_2S$

M: 342 g/mol

Time of reaction: 16h

Yield: 73%

## Synthesis of compound 2

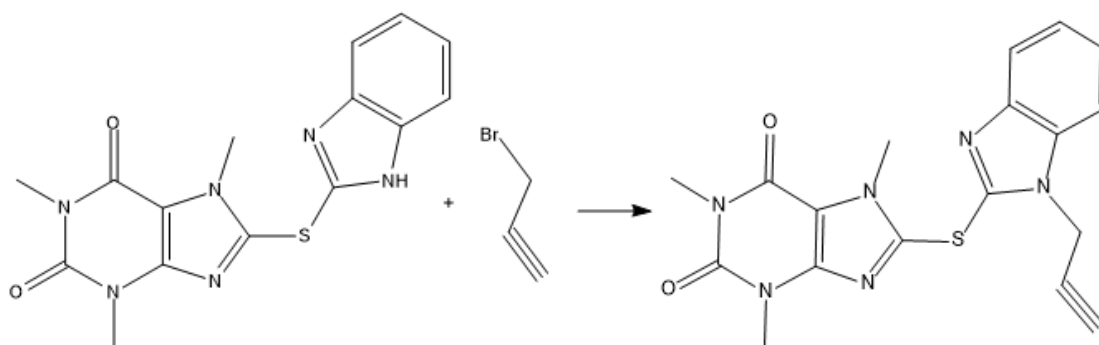


Fig. 6. Scheme of synthesis compound 2

Source: own elaboration

Compound number 1. (180 mg, 0.52 mmol) was dissolved in 3.5 mL of DMSO, then added to solid NaOH (42 mg, 1.04 mmol), then triturated in the flask with a glass rod. It was stirred for 1.5 hours. At this time, propargyl bromide (62 mg, 0.52 mmol) was added. The mixture thickened and dark brown in color. After 1.5 h, 15 mL of water was added. A light brown clay-like solid was obtained. The reaction course is controlled by thin layer chromatography (petroleum ether: ethyl acetate, 3:7).

$C_{18}H_{16}N_6O_2S$

M: 380 g/mol

Time of reaction: 3h

Yield: 60%

## General procedure for the synthesis of compounds 3-9

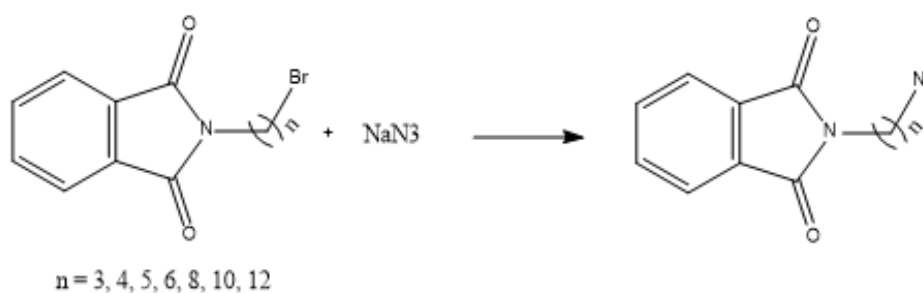


Fig. 7. Scheme of synthesis compounds 3-9

Source: own elaboration

Phthalimide bromide (0.6mmol) was dissolved in acetonitrile (10mL), and sodium azide (2.4mmol) was dissolved in water (10mL). The solutions were combined and stirred for 72h. After completion of the reaction, acetonitrile was evaporated, and the aqueous phase was extracted with ethyl acetate (3x30mL). The combined organic extracts were washed with water (3x30mL) and brine (90mL). The extract was dried over Na<sub>2</sub>SO<sub>4</sub> anhydrous. The solvent was then evaporated. The obtained liquid was crystallized in the air within 48 hours. The reaction was controlled by TLC (2:1 hexane: ethyl acetate plates were burned with 10% H<sub>2</sub>SO<sub>4</sub>).

Time of reaction: 72h

Yield: 95-99%

### General procedure for synthesis of compounds 10-16

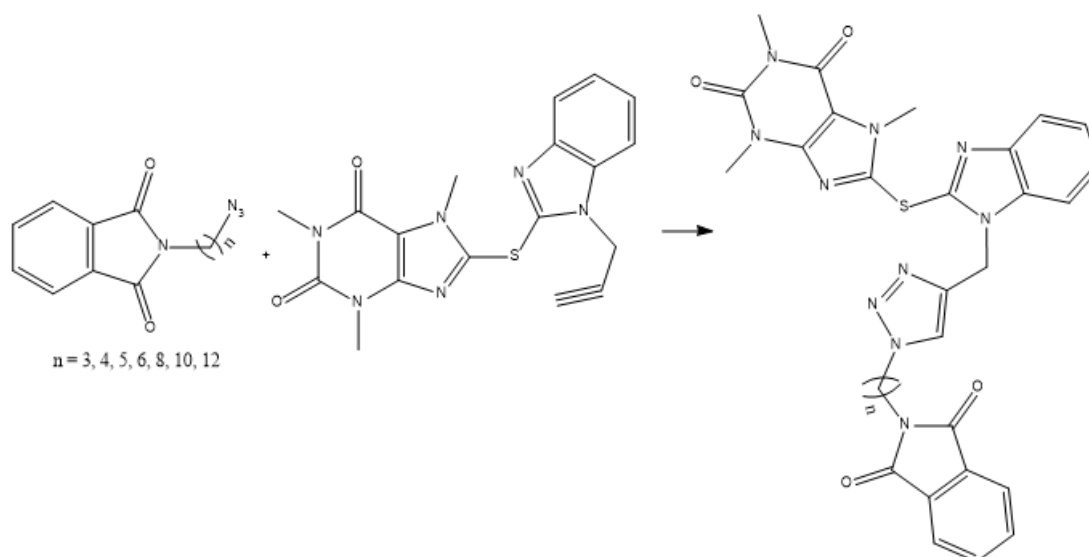


Fig. 8. Scheme of synthesis compounds 10-16  
Source: own elaboration

Compound 2 (0.24 mmol) was dissolved warm in DMSO (4mL) (60°C), compound 3 (0.24 mmol) was added. The solution was clear. The catalyst (CuSO<sub>4</sub>: sodium ascorbate 1:2) was then added portionwise. After completion of the reaction, the mixture was extracted with ethyl acetate (3x25mL). The combined extracts were washed with water (3x25mL) and brine (75mL). The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated. An oily liquid was obtained, which crystallized over time. The reaction was monitored by TLC (petroleum ether: ethyl acetate 3:7).

Time of reaction: 0,5h

Yield: 40-53%

### Results and discussion

As a result of the research, seven new caffeine derivatives with potential biological activity were obtained. The conducted analyzes allowed us to define and confirm the predicted structure of these compounds. The <sup>1</sup>H NMR spectrum of compound 16 is presented below.

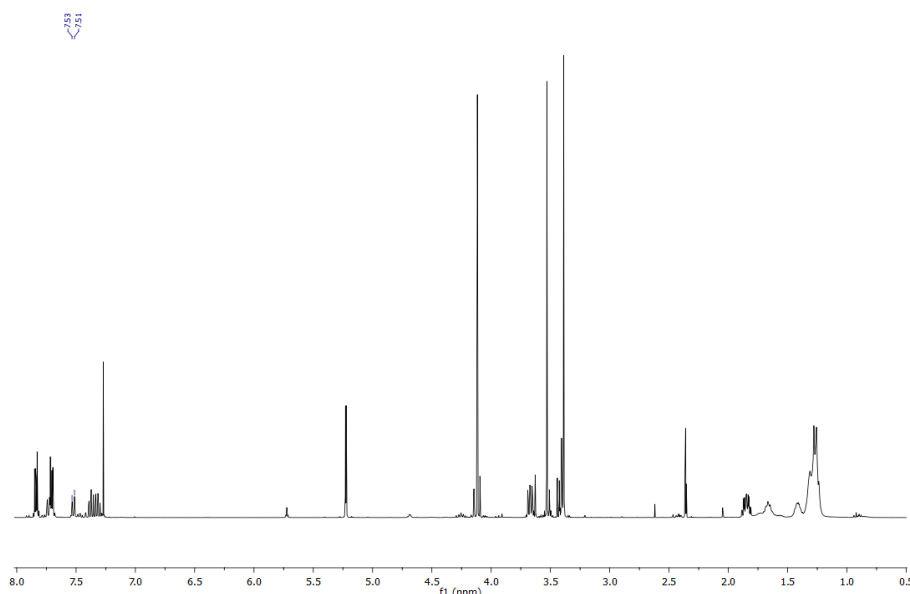


Fig. 9.  $^1\text{H}$  NMR spectrum of compound 16  
Source: own elaboration

The above spectrum shows the signal from the proton in the triazole ring at 7.53 and 7.51 ppm (doublet), Fig. 9.

By quantum chemistry computation, the energy of individual molecules can be determined, and the structure of the molecule can be optimized. An example of an optimized molecule for a compound 10 is shown below.

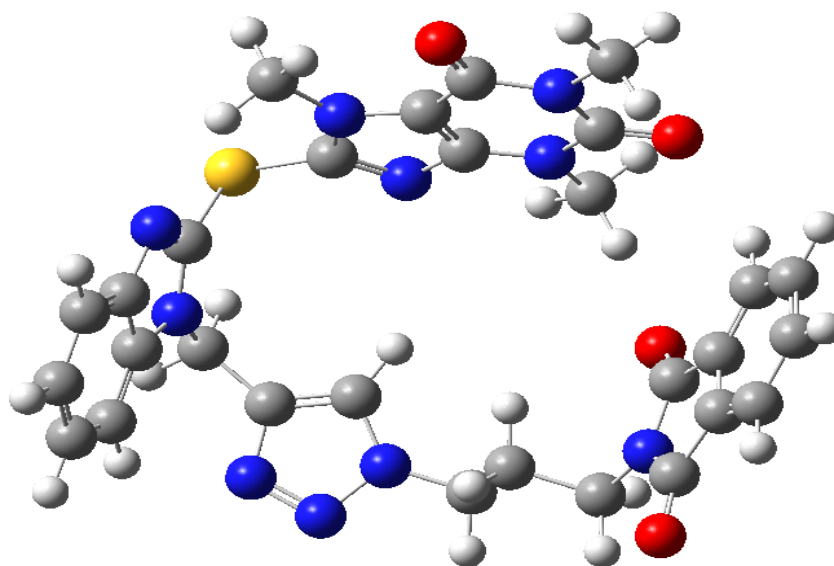


Fig. 10. Optimized molecular structure of compound 10  
Source: GaussView 6.0

If biological tests confirm the potential activity of the compounds obtained, these substances can be used in medicine or as plant protection products.

The click chemistry is fast, does not require harsh reaction conditions and allows us to combine many different molecules.

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**PROMOVENDI**

**Oferujemy:**

- skład i łamanie tekstu,
- wydruk książek abstraktów i monografii z numerem ISBN,
- oprawę graficzną wydruków,
- organizację konferencji,
- pomoc w organizacji konferencji,
- obsługę informatyczną i administracyjną konferencji.



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