9. Annotation of materials following Article 65 (1) of The Regulations for the DEVELOPMENT OF THE ACADEMIC STAFF of The Paisii Hilendarski UNIVERSITY OF PLOVDIV, including self-evaluation of contributions

of

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in connection with participation in a competition for the academic position of "Associate Professor"
in the field of higher education
4. **Natural sciences, mathematics, and informatics**,
professional direction 4.2. **Chemical sciences**,
scientific specialty **Organic chemistry**, **Bioorganic chemistry**,
issued in the State Gazette, No. 98/19th November, 2024

I. Compliance with the requirements for holding the academic position of "Associate Professor" as defined in Article 65 (1) of the regulations for the development of the academic staff of the Paisii Hilendarski Plovdiv University.

I defended my PhD thesis in Organic chemistry in 2015. Since 2021, I have been teaching at the Department of Organic Chemistry, the Paisii Hilendarski Plovdiv University.

I have participated in a number of scientific projects funded by the Ministry of Education, the Science Fund of the University of Food Technology - Plovdiv, and the Scientific and Project Activity Department of the Paisii Hilendarski Plovdiv University.

I present 24 scientific articles, published in journals referenced by Scopus and/or Web of Science, with a total impact factor IF = 54.232. The presented articles were not used for the educational and scientific degree "Doctor" or the academic position "Chief Assistant Professor".

In my scientific research, I have worked in two main scientific directions:

1. Design, synthesis, spectral, and structural characteristics of novel styrylquinolinium compounds with non-linear optic properties and evaluated *in silico*, *in vitro* и *ex vivo* anti-inflammatory activity.
2. Biological activity of propolis and evaluation of its pharmacology profile.

1. DESIGN, SYNTHESIS, SPECTRAL, AND STRUCTURAL CHARACTERISTICS OF NOVEL STYRYLQUINOLINIUM COMPOUNDS WITH NON-LINEAR OPTIC PROPERTIES AND EVALUATED *IN SILICO*, *IN VITRO* И *EX VIVO* ANTI-INFLAMMATORY ACTIVITY.

1.1 INTRODUCTION

Nonlinear optical (NLO) materials are widespread and significantly influence today's daily life. The NLO phenomenon can be described as the intense interaction of laser light's electromagnetic field with materials and the detection of a qualitative change in the incident light's phase, frequency, and polarization [1]. The benefits of organic NLO materials over inorganic ones have garnered a lot of interest. Polymers, molecular crystals, organometallic compounds, and innovative hybrid organic/inorganic nanocomposites are examples of organic NLO materials. These materials' active optical qualities and comparatively inexpensive cost encourage the development of novel NLO dyes [2-4].

Articles submitted for this "associate professor" competition are mostly concerned with the synthesis of molecular organic compounds with NLO characteristics. Particular attention was paid to the synthesis of styrylyum dyes due to their high optical nonlinearities.

In addition to their use in signal processing devices, ultrahigh-speed optical communication, data storage, optical limiters, logic circuit devices, optical switches, optical computers, ultrashort pulse lasers, sensors, laser amplifiers, and terahertz technologies in security screening, substance detection and analysis, medical imaging, atmospheric monitoring, terahertz communication, astronomical research, terahertz radar, etc., these materials are of great scientific and technological interest due to fundamental research on topics like charge transfer, conjugation, polarization, and crystallization in centrosymmetric and noncentrosymmetric lattices, as well as the interaction of matter with light. [5–9].

By creating organic molecules with high values of the initial hyperpolarizability β, it is possible to obtain effective second-order nonlinear susceptibility $\chi 2$ in non-centrosymmetric organic crystals or compounds embedded in polymer matrices and placed in thin layers. In order to maximize β, organic NLO materials are designed using push-pull π-conjugated molecules with large dipole moments. When designing chromophores with high second-order nonlinear optical values, the following factors need to be considered: the material's polarizability; the asymmetric charge distribution with suitable strong donor and acceptor groups; the length of the π-conjugated bridge; and the asymmetric arrangement in the crystal packing, which ultimately determines high values of $\chi2$ in the material. One possible explanation for these materials' high NLO susceptibility is that the π-electron cloud in these systems may be highly polarizable. A nonlinear response of the medium and, as a result, a modulation of its refractive index are caused by the significant charge displacement that occurs when tiny electric fields are applied. This type of polarization is totally electronic, which is necessary for the occurrence of very quick NLO processes. Styrylyum salts are among the most thoroughly researched compounds with NLO characteristics, and significant efforts have been made to create new molecules with these characteristics [11–13]. In addition to being measured experimentally [14,15], the parameters of the second-order NLO, the first hyperpolarizability β and the second-order nonlinear susceptibility $\chi2$, can also be indirectly estimated using spectral and structural analysis techniques [15]. This gives an estimate of the NLO potential of a particular compound.

In addition, styrylpyridinium and styrylquinolinium dyes have demonstrated antibacterial qualities [16,17], certain pharmacological potential, a biological function in plant defense [18], and can be used as biosensors [19,20].

1.2. SYNTHESIS

In the current competition, the synthesis, spectral, and structural characterization of six dyes with NLO activity **1–6**, namely (E)-1-butyl-4-(4-hydroxystyryl)quinolinium iodide (**1**), (E)-1-butyl-4-(4-(dimethylamino)styryl)quinolinium iodide (**2**), 4-{(E)-2-[4-(hydroxy)naphthalen-1-yl]ethenyl}-1-propylquinolinium iodide (**3**), 4-{(E)-2-[4-(hydroxy)naphthalen-1-yl]ethenyl}-1-octylquinolinium iodide (**4**), 4-{(E)-2-[2-(hydroxy)naphthalen-1-yl]ethenyl}-1-octylquinolinium iodide (**5**) and (E)-4-(2-(2-(hydroxy)naphthalen-1-yl)ethenyl)-1-methylquinolinium iodide 4-Methylbenzenesulfonate (**6**) are presented. The compounds were synthesized by Knoevenagel condensation (Cope variant) between 1-alkyl-4-methylquinolinium halide/tosylate as an acceptor moiety precursor and 2-hydroxy-naphthaldehyde, 4-hydroxynaphthaldehyde, 4-hydroxybenzaldehyde, or 4-N,N-dimethylaminobenzaldehyde as "donor" moiety precursors in the presence of a piperidine/glacial acetic acid catalyst [B.4.1.–B.4.5.].

One-dimensional dipoles with donor and acceptor parts at the two opposite ends of the π-conjugated system [21] contribute to the large dipole moments of the molecules and thus the possibility of polarization [22], which results in high values of the first hyperpolarizability β [23]. The general approach for the synthesis of possible NLO dyes was adhered to in the design and synthesis of the compounds in order to make them readily polarizable. In order to boost the molecular asymmetry and, consequently, the second-order nonlinear optical response, donor and acceptor structural components were used in the construction of the NLO-phores [24]. In the present examples, the quinoline fragment was chosen as an electron acceptor [25], while N,N-dimethylamino and the phenolic hydroxyl group as electron donor groups [21].

1.3. STRUCTURAL STUDIES [B.4.1., B.4.6.]

Single crystals of two of the dyes **1** and **2** were grown by the slow evaporation technique from a dilute methanol/2-propanol solution. Dye **1** crystallizes in the monoclinic system, and **2** - in the triclinic, and their space groups are P21/n and P-1, respectively. The unit cells of **1** and **2** contain four, resp. two cations and the equivalent number of halide anions.

The length of the carbon-carbon bonds in the molecular skeletons of both dyes is intermediate between the typical C−C single (1.54 Å) and C=C double (1.34 Å) bonds. The bond lengths in the bridging system of the two cations are as follows: **1**: C11–C10 (1.451), C10=C9 (1.335), C9-C4 (1.453); 2: C11–C10 (1.433 Å), C10=C9 (1.353 Å), C9–C4 (1.406 Å). The high degree of delocalization in the π-electron system of the chromophore is shown by this propensity to equalize the bond lengths, which has also been seen in another dimethylamino substituted dye that has been described in the literature: C5–C6 (1.438 Å), C6=C7 (1.351 Å), and C7–C8 (1.437 Å) [26]. The double bond configuration in both dyes is E(trans), which is consistent with the torsion angle values (about 180º).

The estimation of possible second-order optical nonlinearity at the molecular level and the determination of structural parameters, specifically planarity and the BLA parameter (bond length alternation), are made possible by single crystal X-ray diffraction. Structures that are readily polarizable are linked to high second-order optical nonlinearities [27]. The planarity of these molecules is therefore crucial because it promotes the efficient delocalization of π-electrons, which facilitates their polarization from the donor to the acceptor part of the molecule. This promotes optical nonlinearity [28] and is crucial for the molecular-level estimation of the first hyperpolarizability β.

The chromophore of the dye **1** is practically planar. The plane of the bridging C=C double bond contains the benzene nucleus. The measured torsion angles, which are near 180˚, support this. Concerning the plane of the double bond, the quinoline nucleus exhibits a minor variation of −3.02º. Chandra et al. [29] reported a similar variation. The benzene nucleus in the fused system (quinoline ring) is 3.88 degrees away from the pyridinium ring in the same plane. Weak intermolecular interactions between the parallel chromophores are most likely the cause of these negligible variations. Two identical connections are formed by two parallel molecules. A carbon atom of the bridging double bond engages in a weak intermolecular interaction with a carbon atom of the benzene nucleus, while a carbon atom of the quinoline nucleus makes contact with a carbon atom of the benzene fragment.

A variation of roughly 10˚ is seen in the cation of **2**. This is because the benzene nucleus and the quinoline nucleus are twisted by 8.84˚ and -1.34˚, respectively, with respect to the plane of the double bond. The connections that occur between the dye's cations are most likely the cause of the deviation from planarity. The aromatic sections of the two cations do not overlap; instead, a slip is seen, where the quinoline ring is above the bridging double bond and the benzene portion of one molecule is above the butyl tail of the parallel molecule. The sum of the three C−N−C bond angles (in dye **2**) involving the amine nitrogen, 116.6°, 122.8°, and 120.3°, is 359.7°. One of the dimethylamino group's methyl groups is coplanar with the aromatic nucleus, whereas the other one deviates from planarity by roughly 7°, according to the torsion angles, which are 172.8° and 179.95°. Similar findings were reported by Mishra et al. [30]. One of the methyl groups in dye **2** deviates because it makes two contacts (2.858 Å and 2.386 Å) with an iodide anion and the methyl group of a parallel molecule.

The parallel dye molecule layers of crystal structure **1** are oriented at a specific angle to produce zigzag chains, which are further held up by contacts. Intermolecular interactions also stabilize the layered crystal structure of **2**. The observed connections in the three crystal forms have been used to try and explain the deviation from planarity. On the other hand, these intermolecular interactions and hydrogen bonds stabilize the crystal structures, which also explains why dyes **1** and **2** have been shown to have high melting points. Furthermore, their high melting points are crucial for their technical use in the future.

A number of studies showed that the effective conjugation of π-electrons is not violated by deviations from planarity of up to 8°. The empirically measured value of the initial molecular hyperpolarizability β of identical molecules confirms this [31].

The structural parameter BLA was determined using the information from the X-ray structural analysis. This bond is significant for molecules that can be seen as a superposition of two forms − a neutral (quinoid) form and a zwitterionic (betaine) form, such as previously described compounds. When dye reaches its maximum value in the neutral and zwitterionic states, then BLA and the initial hyperpolarizability β have a good correlation [32,33]. NLO properties are not present in molecules with a BLA value of zero [34]. Teshome et al. discovered that the ideal BLA value, at which the greatest values of the dynamic first hyperpolarizability (βzzz) and the static first hyperpolarizability (βzzz,0) are produced, is 0.1 Å [35].

In our studies, the structural parameter and bond lengths in the π-bridge system of dyes **1** and **2** were compared to structures, namely **DMAQTS** (BLA = 0.099 Å) and **DMAPTS** (BLA = 0.113 Å), having optimal structural parameters and high values of the NLO parameters [35]. The value of the structural parameter BLA for both synthesized dyes was calculated according to Murugan et al. [15] and is 0.117 Å for **1** and 0.067 Å for **2**, respectively. The calculated BLA value for **1** is 0.018 Å more than the BLA of the **DMAQTS** and is comparable to **DMAPTS**. Therefore, the BLA of **1** is near the ideal value and suggests that the degree of charge transfer is significant. This suggests that dye **1** could be a viable option for NLO applications. BLA of **2**, on the other hand, was found to be 0.046 Å and 0.032 Å lower than **DMAPTS** and **DMAQTS**, respectively [35]. It does, however, correspond with that of **TMIPI** [34], which has a pyridinium residue and an indolyl positioned between two bridging double bonds. According to the authors, the two aromatic fragments deviate from the bridging system's plane by roughly 9°. This outcome is in line with the benzene nucleus's reported torsion in 2 and could have an impact on how the electronic charge is distributed across the π-conjugated bridge, which would lower the BLA's value. Therefore, these results suggest a decrease in the optical nonlinearity, due to the linear relationship between BLA and β [36].

Although the molecular nonlinearity of the chromophore only results in even-order susceptibilities χ2 for the crystalline material if the chromophores are oriented asymmetrically, it is known how to model dipolar organic molecules at the molecular level in order to maximize the first hyperpolarizability β [37]. Approximately 75% of all organic compounds do not have second-order optical nonlinearity because they crystallize in centrosymmetric space groups [38].

The parallel orientation of the molecules is a prerequisite for the appearance of macroscopic second-order susceptibility χ2, or second-order NLO effects, in a crystal or material. This results in a macroscopic second-order effect χ2 and the sum of each molecule's microscopic polarization [39, 40]. Push-pull chromophores have a strong inclination to arrange themselves in the crystal in a centrosymmetric fashion due to the great magnitude of molecular dipole moment, and no macroscopic second-order response χ2 is detected [39]. Using crystalline materials has various benefits, including great thermodynamic stability (lack of relaxation) and a high density of chromophores per unit volume.

The cations in the crystals form face-to-face molecular pairs, linked by many contacts and strong π-π interactions, as demonstrated by structural investigations of dyes **1** and **2**. This results in centrosymmetric crystal structures or antiparallel molecular orientation. In these situation, assessing the push-pull chromophores' NLO potential at the molecular level is adequate, therefore this restriction is not insurmountable. Low-pressure organic vapor deposition (LP-OVPD) on amorphous TiO_2 [42], co-crystallization with optically active compounds [38], and Langmuir-Blodgett films [41] are examples of sufficiently effective techniques that allow the molecules to be oriented in parallel (noncentrosymmetric) and utilize the material's NLO capacity.

Since all formed crystals crystallize centrosymmetrically, **push-pull molecules of the styryl dye type exhibit possible NLO properties** at the molecular level that can be proven using thin-layer deposition techniques.

Study of thin layers of dye 4 obtained by pulsed laser deposition [B.4.2.]

High values of the second-order NLO response were experimentally recorded after layers of dye **4** were created using the high-power UV N_2-laser thin-layer deposition (PLD) technology, which was initially used on a styrylquinoline dye [43]. The study focuses on the surface shape and chemical composition of the thin layers produced using this method. The infrared spectra of dye **4** and dye **4** deposited by PLD (pulsed laser deposition) were compared. The majority of the bands in FTIR spectra of the dye were discovered to be retained in the spectrum of the dye's thin layer that was deposited. One asymmetric band appears in the dye layer rather than three or two bands in the starting compound and some bands in the FTIR shift by up to 10 cm^{-1} as a result of the deposition. Degradation processes of a small portion of the deposited dye are linked to the formation of two bands at 1508 cm^{-1} and 1149 cm^{-1}.

Atomic force microscopy surface examination reveals that the deposited films have a layered structure and a highly ordered uniform shape. The films' suitability for nonlinear optical applications depends on their degree of homogeneity and surface roughness. The current results, in conjunction with earlier research [43], offer the possibility of obtaining styrylquinolinium dye thin films for direct photonic applications [B.4.2.].

1.4. SPECTRAL STUDIES

1.4.1. INFRARED SPECTROSCOPY [B.4.1. - B.4.4.]

Vibrational techniques can give important insights into the force field of molecules and can help to a thorough understanding of their physical and chemical characteristics. Solid-state effects like Evan's holes and Davydov splitting of the bands, which are typical of salt-like compounds, can be studied using the infrared spectra of styrylquinolinium dyes [44].

The O-H stretching vibration υ(OH) is represented by a band in the range 3435–3419 cm^{-1} found in the infrared spectra of dyes with a phenolic hydroxyl group. The OH group's involvement in hydrogen bonding and contacts causes the band in all dyes under consideration to move by roughly 200 cm^{-1} to lower wavenumbers. The description of crystal structure of **1** provides support for this claim. It is seen that the phenolic hydroxyl group contributes to the iodide anion's hydrogen bond creation.

The bands that correspond to the υ(P-N) stretching vibrations of the methyl and methylene groups of the alkyl tails, which are connected to the quaternary nitrogen atom, are found in the interval 2995-2850 cm^{-1}, while the υ(P-H) stretching vibrations of the aromatic nuclei and the bridging double bond are found in the interval 3113-3014 cm^{-1}.

A complicated infrared spectral band in the 1800–400 cm^{-1} range characterizes dyes with intramolecular charge transfer, like the ones under study. Quantum chemistry simulations have demonstrated that most of the bands in this region are due to mixed vibrations [26]. This behavior is caused by a substantial delocalization of charge in the dye's cationic portion, which interacts with the compound's donor and acceptor sections electronically and vibrationally. As a result, the bands associated with the corresponding normal vibrations are seen to move to lower wave numbers, and some of them become more intense.

In contrast to stilbene, the bands in the infrared spectra of the dyes under study are asymmetric, which is one of their distinguishing characteristics [45]. The crystal field effect and the charge transfer complex are the causes; the stronger the latter, the more asymmetric the bands are [26]. [B.4.1.–B.4.4.]

The dyes' NLO properties depend on the extent of charge transfer. The degree of charge transfer was shown to be influenced by the length of the alkyl chain, which is attached to the quaternary nitrogen atom in the quinoline nucleus.

The IR spectra of four dyes—methyl, ethyl, n-propyl (4), and n-butyl—with the same donor moieties (4-hydroxynaphthyl) but distinct alkyl chains at the quaternary quinoline nitrogen atom were compared with one another and with the theoretical spectrum of one dye, three of which have known syntheses [46]. It was investigated how much the charge transfer would change if the positive ion's electron deficit was reduced. It should be mentioned that the experimental IR spectra data for the various dyes have numerical values that are near to one another and within the error. The most likely explanation is that the chromophore's N-alkyl group has a negligible impact on the charge transfer complex (weak +I action). [B.4.3.]

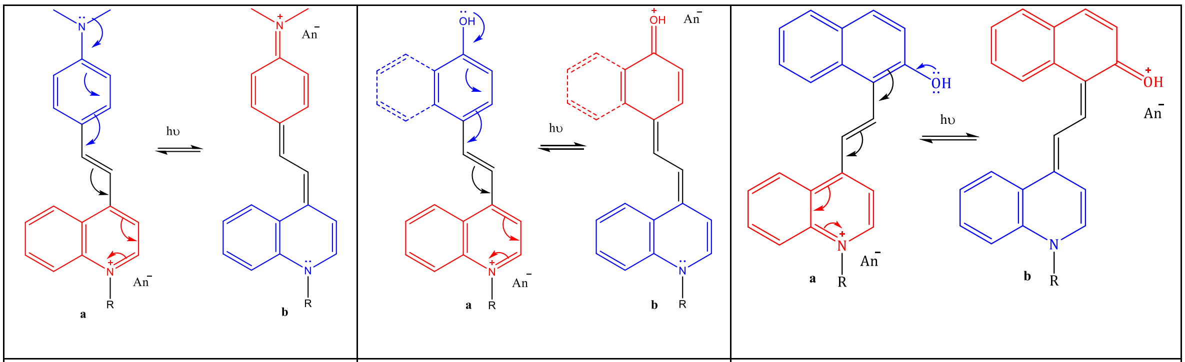
OH and N,N-dimethylamino substituted dyes' infrared spectra were mapped. Vibrational spectroscopy was used to identify the charge transfer complex. Vibrational spectroscopy revealed that the charge transfer in the investigated 4-hydroxystyrylquinolinium dyes is barely affected by the decrease in the electron deficiency at the quaternary quinoline nitrogen atom brought on by the alkyl chain's growing positive induction effect.

1.4.2. UV-VISIBLE SPECTROSCOPY [B.4.1. and B.4.4.]

The S_0 – CT transition (where S_0 is the ground singlet state and CT is the charge transfer state) is represented by an intense absorption band in the visible range, with a shift based on the solvent used, according to the examination of the absorption spectra. The intramolecular charge transfer from the electron-donating group (NMe2, OH) to the quaternary nitrogen atom in the quinoline moiety causes the absorption. All of the dyes' spectra show an absorption at roughly 300 nm caused by the π–π^* transition in addition to the CT band.

The dyes' UV-visible spectra in variously polar solutions were examined. In addition to the typical solute-solvent interaction, the solutions exhibit a particular interaction that involves the formation of charge-transfer complexes, hydrogen bonds, and molecular complexes, depending on the solvent's polarity. This interaction causes a shift in the equilibrium excited state level to higher or lower energy by altering the energy difference ΔE between the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO).

The excited (quinoid form) and ground (benzoid form) states of the three primary chromophores are schematically depicted in Figure 1. In each of the several forms, the chromophore's positive charge is located on a distinct kind of heteroatom as it transitions from the quinolinium residue to the amino/OH group following excitation. It is located at the quinolinium nitrogen in the ground state (a) and the imine nitrogen or oxygen in the excited state (b).



а) **2** б) **1 и 3** в) **5**

Figure 1. Ground state (benzoic form) (a) and excited state (quinoid form) (b) of the three main types of chromophores in a) N,N-dimethylamino dyes **2**; b) 4-hydroxy dyes **1** and **3** and c) 2-hydroxy dye **5**.

While the hydroxy-containing dyes **1** and **5** had two or three absorption bands in the majority of the investigated solvents, with the exception of water, the UV-visible spectra of dimethylamino dye **2** have only one maximum that corresponds to the CT band. The benzoic and quinoid versions of the two hydroxy dyes (1 and 5) exhibit distinct bands in their UV-visible spectra. The monomer of the quinoid form is found between 662 and 694 nm, while the benzoic form of both dyes in the various solvents is found between 414 and 481 nm. In these circumstances, dimers show up as a shoulder of the monomer band moved to shorter wavelengths in the 580–648 nm range, and the quinoid form shows a propensity to aggregate. Solvents can stabilize both the benzoic and quinoid forms in different ways, depending on how polar they are. As a result, it's possible that some people only have the benzoic or quinoid forms, or that both are in balance. Only the benzoic form of hydroxy dyes in water is observable in their UV-visible spectra. Both colors exhibit the emergence of bands for the benzoic form and the quinoid dimer in the solvent methanol. The solvents ethanol and 1-butanol also exhibit bands for these dye 1 variants.

Three bands in dye **1**, benzoic, quinoid, and dimer of the quinoid form, are detected in pyridine, acetone, and dimethyl sulfoxide, whereas bands in **5** are registered in acetonitrile, acetone, chloroform, and benzene. Only the quinoid form has bands in the spectra of **5** for the solvents ethanol and dimethylformamide. The two variants of dimethylamino dye **5** do not exhibit two distinct bands in their UV-visible spectra; instead, they appear as a single widened band in the 512–568 nm range, with a shift based on the solvent type. The spectral shift of the absorption maximum of the CT band due to a change in solvent polarity from water to chloroform for hydroxy dye 5 is 248 nm. The difference in the maximum values observed in solvents 1,2-dichloroethane and water for dyes 1 and 2 is 262 nm and 75 nm, respectively. A significant shift of the CT band from nonpolar to polar solvent is observed in the group of hydroxy dyes (1 and 5) compared to dimethylamino dye 2. The observed solvatochromic behavior is associated with a change in the dipole moment upon electronic excitation and interaction of the dipole moment with the polarized environment [47], and the reported large charge shift is indicative of the possibility of high polarizability [48,49]. The value of β can be determined by an indirect estimate of Δμ (the difference in the dipole moments of the ground and excited states) using the influence of solvent polarity on the absorption spectra [50]. The dyes studied were found to possess reversible solvatochromism [B.4.1. and B.4.4.]. The value of β can be determined by an indirect estimate of Δμ (the difference in the dipole moments of the ground and excited states) using the influence of solvent polarity on the absorption spectra [50]. The dyes studied were found to possess reversible solvatochromism [B.4.1. and B.4.4.].

Based on the information from the UV-visible spectra, the difference in the energies of the ground and excited states ΔE of three dyes with the same acceptor moiety and different donor moieties - hydroxyl at the 4- and 2- positions (5), as well as an N,N-dimethylamino group at the 4-position in the naphthalene nucleus, two of the dyes are known [46]. The lowest values of ΔE were calculated for the two hydroxy dyes [B.4.4.]. For these three dyes, a reversible electrochemical behavior (oxidation/reduction) was also demonstrated. The dimethylamino dye undergoes this process the slowest, while the two hydroxy dyes undergo it the fastest. Additionally, possible mechanism is suggested [B.4.4.].

1.4.3. SPECTROSCOPY OF FLUORSENCE

In solvents with varying polarities, the emission characteristics of dyes **1** and **2** were examined. It was discovered that **2** does not display its own emission, whereas **1** shows faint fluorescence. Since it has been demonstrated that styryl dyes give objects the ability to glow, the absence of fluorescence or weak fluorescence is not a drawback. This property has been effectively used for bioimaging of the plasma membrane of both plant and animal cells, including tissues [51]. Styryl dyes have been shown to exhibit a 28-fold increase in fluorescence emission when they attach to tau clumps, suggesting the possibility of developing novel fluorescent probes for Alzheimer's disease early detection [52]. **[B.4.1]**

1.5. IN VITRO ANTI-INFLAMMATORY ACTIVITY [B.4.5]

Research on styryl dyes has recently received more attention; specifically, an investigation of their biological activity has been conducted. It has been demonstrated that (E)-4-(2-(2-(hydroxy)naphthalen-1-yl)ethenyl) 4-methylbenzenesulfonate **6** has good anti-inflammatory properties. based on an in vitro test to evaluate the decrease in albumin's thermal denaturation. According to the findings, the compound's IC_{50} is 350 μg/mL, but diclofenac's IC_{50} is 471.3 μg/mL. This indicates that dye **6** has a stronger anti-inflammatory effect. The molecular docking process validates the in vitro data and explains them by suggesting that the dye molecule may engage with the albumin molecule's amino acid residues through a variety of hydrogen bonds, hydrophobic interactions, and Van der Waals forces, stabilizing the albumin molecule. By keeping albumin from becoming denaturated during inflammation, these interactions help to maintain its integrity. According to the results of the histochemical studies, compound **6** can decrease inflammatory processes. Additionally, its antioxidant activity was assessed.

CONTRIBUTIONS

I. Scientific contributions.

1. Design of dyes as NLO-phores and optimization of synthetic approaches for their synthesis.

2. Investigation of the impementation of various structural elements (donor or acceptor groups) in order to influence the charge transfer in the system and increase the second-order nonlinear optical response.

II. Synthetic and applied contributions

1. Six novel styrylquinolinium dyes were synthesized.

2. Three compounds were characterized by single crystal X-RAY diffraction

3. Thin films of one of the studied dyes deposited using the PLD technique were successfully deposited. The chemical composition and surface of the deposited layers were investigated.

4. The IR spectra of OH and N,N-dimethylamino substituted styrylquinolinium dyes have been mapped in details in order to prove charge transfer.

5. The UV-visible spectra of hydroxy and dimethylamino dyes in solvents of different polarity were measured. The absorption bands were assigned to the corresponding electronic

transitions, and the behavior of the band corresponding to the intramolecular charge transfer state (CT band) was studied.

6. A significant shift of the CT band from a nonpolar to a polar solvent was established for hydroxy dyes **1** (1,2-dichloroethane / water) and **5** (chloroform / water). The studied hydroxy dyes (**1** and **5**) demonstrate better charge transfer compared to dimethylamino dye **2**.

7. Reversible electrochemical behavior (oxidation/reduction) of three dyes was evaluated. The influence of the nature of the donor moieties has also been established.

8. A quantitative approach for evaluating the inhibition of albumin denaturation, an indicator of in vitro anti-inflammatory activity, was developed. Ex vivo evaluation of anti-inflammatory efficacy and in silico computations were used to validate the approach.

III. Theoretical contributions

1. Based on the UV-visible spectra, the energies of the ground and excited states were calculated, as well as the energy difference between the levels of three dyes with the same acceptor moiety and different donor moieties: hydroxyl at 4- and 2-positions and N,N-dimethylamino group at 4-position in the naphthalene core.

2. The lower the $\Delta E_{HOMO/LUMO}$ value, the easier and more favorable the charge transfer is.

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2. BIOLOGICAL ACTIVITY OF PROPOLIS AND EVALUATION OF ITS PHARMACOLOGY PROFILE

One of the most significant and distinctive bee products is propolis, or bee glue, which has a variety of biological qualities. Honey bees (Apis mellifera L.) generate this sticky, resinous substance, which is mostly utilized as construction material in their hives. Because of its strong antibacterial qualities, propolis is regarded as a component of the bees' immune system and is thought to be crucial to their social immunity. It offers the colony protection against parasitic and infectious disorders as well as general prophylaxis [1].

Propolis is a multicolored, complex bioproduct that might be green, red, yellow, or brown. Depending on the plant source, season, climate, altitude, and other features of the geographic area from which it is sourced, its chemical makeup and biological traits might differ considerably. Recent studies have shown that propolis contains polyphenols, primarily flavonoids, which are among its primary bioactive constituents. Propolis contains additional biologically active substances, including aliphatic and aromatic acids, their esters, hydrocarbons, steroids, enzymes, alcohols, aldehydes, ketones, amino acids, micro- and macroelements, vitamins, isoprenoids, sugars, and more [2,3].

The strong biological activity and medicinal qualities of propolis are determined by its rich chemical composition. It is used extensively in medicine to treat a variety of illnesses due to its antibacterial, antioxidant, anti-inflammatory, antiparasitic, anticarcinogenic, hepatoprotective, antiulcerogenic, antiallergic, antidiabetic, immunomodulatory, anesthetic, and other activities [4,5].

2.1. EVALUATION OF PHARMACOLOGICAL PROFILE OF PROPOLIS

Eighty propolis samples from Bulgaria's 28 regions—which span the country's five climate zones—were the subject of a comprehensive, organized investigation. [B.4.7.] Information about Bulgarian propolis is still limited, despite extensive chemical and pharmacological study on the substance and a large number of recent scientific papers. The research enhances the data on the antibacterial, antioxidant, and physicochemical characteristics of propolis gathered from several Bulgarian locations.

Propolis from several continental regions has been identified by its color features. One of the observable physicochemical traits of propolis is its color, which serves as a crucial predictor of both its biological activity and phenolic content. Depending on the traits and vegetation of the climatic zone from which it is sourced, the raw propolis samples collected from the 28 regions of Bulgaria exhibit a brown or green hue with matching differences in shade, ranging from light to dark. They are gathered. Propolis from the Black Sea, mountain, and continental-Mediterranean climate zones is brown, while propolis from the temperate-continental and transitional-continental climate zones is either brown or green. The proportion of two propolis colors is as follows: 20% green and 80% brown in the transitional continental zone, and 29.3% green and 70.7% brown in the temperate continental zone.

Propolis's pH and moisture content are physicochemical characteristics that are strongly influenced by local plant diversity, temperature, and air relative humidity. The findings demonstrate that, irrespective of their place of origin, the pH and moisture content of the propolis samples under investigation fluctuate within very small ranges. The range of pH values is 4.82 to 5.87. The moisture content falls between 0.98% and 2.97%, which is consistent with data from the literature. The biological activity of propolis, particularly its antioxidant capacity, is attributed to a diverse range of chemical components, primarily polyphenols (flavonoids, phenolic acids, and their esters).

It was discovered that the total phenol concentration varied greatly between propolis samples, ranging from 63.14 mg GAE/g to 737.27 mg GAE/g. Additionally, the total flavonoid concentration ranged greatly from 29.22 mg QE/g to 234.17 mg QE/g. The greatest levels of total flavonoids were found to correlate with the highest levels of total phenols in the majority of propolis samples. Resins from poplars and other broad-leaved tree species, which determine the phytochemical profile of the poplar type of propolis, are the main plant sources in Bulgaria's temperate continental and transitional continental zones, which is why the majority of the studied propolis samples that displayed the highest amounts of polyphenols and flavonoids came from these regions.

Propolis samples' antioxidant activity was assessed using the DPPH and FRAP techniques. According to the DPPH approach, the antioxidant activity of propolis samples ranged from 18.56 mM TE/g to 1598.66 mM TE/g, whereas the results of the second method indicated that the values were between 82.28 mM TE/g and 1208.81 mM TE/g. The highest levels of phenols and flavonoids were correlated with the highest antioxidant values in the majority of propolis samples. The antioxidant activity, total flavonoid content, and total phenolic content were all correlated. The overall phenolic and flavonoid content has a significant influence on the antioxidant capacity.

There was a significant correlation between the two antioxidant methods (r2(DPPH/FRAP) = 0.6806, r2(TPC/DPPH) = 0.6793, r2(TPC/FRAP) = 0.4360, r2(TFC/DPPH) = 0.7760, r2(TFC/FRAP) = 0.5546), and a high correlation between the content of phenols and flavonoids (r2(TPC/DPPH)=0.8462).

The Gram-positive bacteria *M. luteus* 2YC-YT, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *L. monocytogenes* NBIMCC 8632, *L. innocua* ATCC 33090, *B. amyloliquefaciens* 4BCL-YT, *E. faecalis* ATCC 19433, and *E. faecuim* ATCC 19434 were all found to be susceptible to the high antibacterial activity of propolis extracts. Propolis extracts showed moderate antibacterial activity against *S. enteritidis* ATCC 13076, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 9027. Some of the samples had little to no antibacterial activity against *Klebsiella* sp. and *P. vulgaris* ATCC 6380. When tested against *Rhizopus* sp., the majority of the extracts exhibited strong antifungal activity. Propolis extracts showed a moderately inhibitory impact on *Penicillium* sp. and *A. Flavus*, antibiotic activity against the yeasts *S. moniliforme* ATCC 38932, *A. niger* ATCC 1015, and *F. moniliforme* ATCC 38932.

New methods of food production and the use of certain physiologically active substances as biopreservatives have been prompted by the increasing customer demands for minimally processed, fresh food products free of chemical preservatives. Propolis's wide range of antibacterial activity, strong antioxidant qualities, and lack of toxicity make it a fantastic tool for biological preservation and present numerous prospects for use in the food business. Propolis can be included into food biopackaging materials, applied to the product's surface as an edible coating (biofilm), or put straight to the food matrix as an extract.

The necessity of biopreservation and the suitability of propolis as a preservative for meat, sausages, dairy products, cheese, yellow cheese, and perishable fruits and vegetables were determined by a thorough literature analysis. Meat and meat products are extremely vulnerable to microbial deterioration and frequently serve as a breeding ground for pathogenic and saprophytic microbes. Propolis extracts have been shown to directly reduce the growth of food pathogens, including *Salmonella* sp., *Clostridium* sp., *Escherichia* coli, *Staphylococcus aureus*, and *Listeria innocua*, when added to fermented meat products (such as sausages) and fish. Furthermore, the addition of propolis to these products serves as a natural antioxidant source and inhibits the oxidation of lipids during the ripening, processing, and storage stages. By slowing the breakdown of proteins in sausages, propolis enhances their sensory appeal [6–8].

Applying propolis to the surface, either by itself or in combination with other biopolymers (cellulose, pectin, starch derivatives, etc.), can also enhance the quality and shelf life of meat products [9, 10]. Similar to meat and meat products, milk and dairy products are good media for microbial growth and are crucial to the establishment and spread of foodborne diseases. A technological procedure called pasteurization greatly lowers the amount of germs in raw milk before it is consumed or used to make dairy products, but it does not provide a sterile product [11,12]. Therefore, the use of safe and natural preservatives like propolis can help to tackle the issue of undesired microorganisms surviving in milk.

Propolis, on the other hand, can boost the antioxidant capacity of dairy products and stop lipid oxidation and the related formation of aldehydes, as pasteurization lowers their antioxidant activity [13]. Propolis has a strong antibacterial impact on L. monocytogenes and improves the milk's organoleptic properties when added [14,15].

Mold spoiling, which can happen during ripening or storage, can seriously impair the sensory qualities of some dairy products, particularly hard cheeses. It can also result in toxicity to customers and financial losses for producers. Many cheese makers utilize antibiotics, weak acids, and high salt concentrations to prevent the growth of mold. However, the usage of natural antibacterial agents, such propolis, can counteract the adverse health effects of some antibiotics [16].

Mold degradation is one of the most significant issues with fruit and vegetable marketing, storage, and shelf life. The fungi Penicillium sp., Rhizopus sp., Mucor sp., and Botrytis cinerea are the most frequent causes of this. Fruit quality deteriorates, loses its commercial appeal, and offers health risks to consumers as a result of fungal growth. New techniques for biological preservation employing natural antibacterial substances have been developed as a result of the demonstrated harm caused by chemical fungicides used to preserve different crops. Edible coatings, which are thin layers of biodegradable materials (mostly polysaccharides) applied to the fruit surface, are one of the most often used unconventional methods for biopreservation of perishable fruits. Their primary purpose is to shield fruits from physical, chemical, and microbiological harm. This delays microbial deterioration, prolongs the shelf life of citrus fruits (oranges, tangerines, and grapefruit), cucumbers, leafy vegetables, and Muscatel grapes, and lowers financial losses [17–21].

2.2. APPLICATION OF PROPOLIS EXTRACTS IN EDIBLE COATINGS IN BIOLOGICAL PRESERVATION OF FOOD

2.2.1. Application of propolis extract in the composition of edible carboxymethylcellulose coatings for perishable blueberry fruits.

According to the literature research, there is currently a dearth of information in the scientific literature regarding the biopreservation of blueberries, and edible coverings containing propolis have not been extensively investigated in this regard. A control sample (K) was used to compare the effects of applying edible coatings of carboxymethylcellulose (CMC) and carboxymethylcellulose with embedded propolis (CMC+P) on the quality attributes and extension of the shelf life of fresh blueberries during refrigeration. From the 80 samples examined, the propolis type with the highest levels of flavonoids and polyphenols as well as the highest levels of antioxidant and antibacterial activity was chosen [B.4.7.].

It has been demonstrated that propolis extract-based edible coating can successfully shield blueberry fruits from microbiological activity and postpone spoiling. Propolis' potent antibacterial activity and antioxidant qualities are linked to this protective effect. It has been discovered that treating blueberry fruits with an edible coating of 1% CMC solution and 1% propolis extract helps to prolong their shelf life and enhance their overall look by preventing drying out and moisture loss throughout the storage period.

The following indicators were monitored:

Until the end of the storage period, it was discovered that the parameters associated with fruit ripening processes and post-harvest changes, such as the decrease in titratable acidity and the corresponding increase in pH values, were unaffected and remained similar in the three types of samples: K, CMC, and CMC+P. During the first 12 days of refrigerated storage, it was discovered that the levels of total soluble solids (TSS), which is measured by Brix, remained consistent across all experimental groups. Due to the drying of the fruits and the migration of water into the environment, there was a minor increase in TSS levels on the sixteenth day in all experimental groups, and these levels continued to rise progressively until the conclusion of the storage period.

2.2.1.1. Evaluation of the changes in microbiological parameters

Total plate count (mesophilic aerobic and facultative anaerobic microorganisms)

It was found that the number of aerobic mesophilic bacteria and facultative anaerobic microorganisms in the control and CMC-coated fruits increased with the extension of the storage time, reaching the highest values at the end of the observation period (20th) day. Propolis extract in edible coatings reduced the total number of microorganisms, and blueberries treated with CMC with the addition of propolis (CMC+P) retained significantly lower populations of mesophilic aerobic and facultative anaerobic bacteria until the end of the storage period compared to the control and CMC-coated fruits, which is explained by the antimicrobial potential of the propolis extract.

The addition of propolis extract to the composition of the edible coatings (CMC+P) resulted in a significant decrease in yeast counts, especially after the eighth day of storage compared to the control fruits and the carboxymethylcellulose-treated fruits, and this trend continued until the end of the observation period. It was discovered that during the 20-day storage period of the three types of samples at 4°C, a trend of increasing yeast and fungal counts was observed in both the control group and the CMC-coated fruits.

The higher inhibitory effect of propolis extract on fungal growth was measured after the 8th day of refrigerated storage, visibly expressed by a decrease in the incidence of rot compared to the uncoated and treated fruits.

One promising method for biopreservation and extending the shelf life of blueberry fruits is the use of propolis into food coating formulations. Every study demonstrates that fruits coated with an edible coating containing propolis have less microbial development, indicating the possibility of edible coatings to prolong the shelf life and maintain the nutritional content of fresh blueberries.

2.2.1.2. Changes in total phenolic content (TPC)

Naturally occurring secondary metabolites of plants are called phenolic compounds. They are regarded as biologically active, non-nutritive compounds that assess the antioxidant capacity of various plant species' fruits, leaves, nuts, seeds, flowers, and bark. One of the best sources of substances with antioxidant properties is blueberries [22].

Throughout the entire storage period (days 0, 4, 8, 12, 16, and 20), it was crucial to keep an eye on the levels of polyphenols and antioxidant activity. TPC levels in both control and treated blueberries remained comparatively high over the first four days of refrigeration, approaching the initial polyphenol concentration. All experimental groups showed a progressive decline in quantities after the eighth day, with concentrations of 0.93 mg GAE/g fw (K), 1.01 mg GAE/g fw (CMC), and 1.14 mg GAE/g fw (CMC+P) at the end of the observation period (day 20). When compared to uncoated fruits, coated blueberries (CMC and CMC+P) retained greater TPC values throughout storage. Throughout the storage term, edible coatings enhanced with propolis extract (CMC+P) demonstrated the most protective impact on polyphenol content.

The antioxidant activity in every experimental group progressively declined throughout the course of the 20-day storage period at 4°C, according to the results from the DPPH and FRAP techniques.

According to the DPPH assay results, the antioxidant concentrations in the coated blueberries (CMC and CMC+P) dropped to levels comparable to those in the control fruits after the first eight days of refrigeration, when the application of edible coatings prevented this decline more successfully. According to the FRAP assay results, blueberries with edible coatings (CMC and CMC+P) maintained noticeably higher antioxidant levels over the course of storage than the control. Propolis extract-enriched edible coatings (CMC+P) showed the strongest protective effect on antioxidant activity, extending the fruit's shelf life and boosting its nutritional content and quality.

There was a strong association between DPPH and FRAP, as well as between the antioxidant activity as measured by both techniques and the levels of total polyphenols. Consequently, the amounts of polyphenols in the blueberry fruits under study are correlated with antioxidant activity.

2.2.2. Composition of edible carboxymethylcellulose coatings for Bulgarian yellow cheese using extracts from three species of propolis [B.4.10]

When yellow cheese was stored for 56 days at 4˚ C in a refrigerator, the effects of adding 1% propolis to a carboxymethylcellulose food coating on its properties were investigated. Three samples of yellow cheese with 1% ethanol extract of three different varieties of propolis added to the food coating were compared to the control [B.4.10.]. Among the 80 Bulgarian propolis varieties that were tested, those with varying levels of antioxidant activity, total phenols and flavonoids, and antibacterial activity were chosen [B.4.7.].

It was discovered that the propolis coating from the village of Vladimir reduced the growth of fungi on the cheese's surface, whilst the edible films containing propolis from the cities of Bankya and Simitli prevented the growth. It was discovered that the overall quantity of lactic acid bacteria and yeast in the cheese was unaffected by the application of the three varieties of edible films containing propolis.

Customer feedback is crucial when utilizing propolis in edible casings. Though all three samples are generally similar to the control, the tasting evaluation's results showed that consumers preferred yellow cheese with an edible coating that included propolis from the town of Simitli, followed by the control and, lastly, samples with propolis from the town of Bankya and the village of Vladimir. The findings validate the acceptable sensory qualities of yellow cheese with an edible coating that contains propolis. Therefore, by preventing and delaying the growth of fungus in yellow cheese, the use of edible carboxymethylcellulose casings with propolis extracts contained can be regarded as an efficient biopreservation method, enhancing the product's safety and its shelf life.

SCIENTIFIC AND APPLIED CONTRIBUTIONS

1. Eighty propolis samples from all 28 regions of Bulgaria were examined for the first time.

2. Propolis's physicochemical characteristics, polyphenol and flavonoid content, and, correspondingly, its association with its antioxidant and antibacterial activity were shown to be correlated with the region from which it was obtained.

3. It's been established that the highest amounts of phenols and flavonoids were shown to be associated with the highest antioxidant properties of propolis.

4. It has been demonstrated that propolis extracts have strong antibacterial action against Gram-positive bacteria.

5. It was investigated how edible carboxymethylcellulose coatings containing propolis extended the shelf life of food items. Edible coatings have been shown to efficiently protect the products under study.

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ANNOTATION OF MATERIALS

B.4.1. Todorova M., Seidel R.W., Stoyanova M., Kolev T.M., Bakalska R. *Comparing the crystal structures and spectroscopic properties of a p-hydroxy styrylquinolinium dye with those of its p-dimethylamino analogue*. Heliyon **2024**, *10 (8)*, e29315.
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(IF$_{\mathbf{(2023)}}$ **3.4**, SJR$_{(2023)}$ **0.617**), Scopus (**Q1**) и Web of Science (**Q2**)

Two previously synthesized styrylquinolinium dyes, namely (E)-1-butyl-4-(4-(dimethylamino)styryl)quinolinium iodide (D36) and (E)-1-butyl-4-(4-hydroxystyryl)quinolinium iodide (D34), were compared in terms of their properties by single-crystal X-ray diffraction (XRD), Hirshfeld surface analysis, Fourier transform Raman (FT-Raman), Fourier transform infrared (FT-IR), fluorescence, and ultraviolet–visible (UV–Vis) spectroscopy, and ^{1}H- and ^{13}C-NMR methods. Both dyes D36 and D34 crystallized in the triclinic and monoclinic systems in the centrosymmetric space groups P-1 and P2$_1$/n, respectively. The unit cell of D36 contains two molecules of the dye, participating in weak intermolecular interactions, whereas that of D34 contains four formula units. The phenolic hydroxy group of D34 participates in the formation of a hydrogen bond with the iodide anion. The 4-styrylquinolinium moieties of the cationic dye molecules are nearly planar. The dihedral angle between the mean planes through the ten-membered quinolinium system and the benzene ring is 7.5° in D36 and 5.9(1)° in D34. The structural parameters planarity and bond length alternation (BLA) are discussed, which are important for the evaluation of the first hyperpolarizability β at the molecular level, even in a centrosymmetric crystal. The UV–visible spectra of the dyes in 14 solvents of different polarities were investigated. The reversible solvatochromic behavior of the dyes is demonstrated experimentally and compared with known “binuclear dyes” by evaluating the Rezende model. Dye D36 does not fluoresce, and D34 has a very low emission in the solvents tested.

B.4.2. Sotirov S., **Todorova M.**, Draganov M., Penchev P., Bakalska R., Serbezov V. *Investigation of new stilbazolium dye thin films deposited by pulsed laser deposition*.
Proceedings of SPIE - The International Society for Optical Engineering **2013**, *8770*, 87700E.
DOI: 10.1117/12.2011876
ISSN: 1996756X
ISBN: 978-081949568-6
(SJR$_{(2013)}$ **0.236**). Scopus, Web of Science

In present work we report the analysis of thin films and targets from new stilbazolium dye E-4-(2-(4-hydroxynaphthalen-1-yl)vinyl)-1-octylquinolinium iodide (**D1**) deposited by Pulsed Laser Deposition (PLD) technique using high power UV TEA N$_2$ laser. The thin films are deposited onto substrates – KBr, 316L SS alloy, optical glass and aluminum foil. The films were characterized by FTIR spectroscopy, bright field microscopy, fluorescence microscopy and atomic force microscopy (AFM) analysis. FTIR spectroscopic analysis of thin films and target material shows small differences between deposited films and native substance. The films are found to be homogeneous by AFM results and without any cracks and droplets on the surfaces. The present study demonstrates the ability of PLD technique to provide thin films from new stilbazolium dyes with good quality when they are applied as non-linear optical (NLO) organic materials on different type of substrates.

B.4.3. Todorova M., Bakalska R. *Syntheses and vibrational spectroscopic characteristics of series ionic merocyanine dyes*. Bulgarian Chemical Communications **2018**, *50*, 156 - 164.
ISSN 08619808
(SJR$_{(2018)}$ **0.137**), Scopus (**Q4**)

A series ionic merocyanine dyes with enlarged π-conjugated system and varying length of the N-alkyl chain were synthesized and investigated by means of solid-state IR and Raman spectroscopy. Quantum chemical calculations at the DFT level were performed to predict electronic structure and vibrational data. Nearly all IR bands are asymmetric. As a result of electronic interaction due to the intramolecular charge transfer (ICT) which leads to vibrational one, nearly all vibrations are strongly mixed and the intensities are strong influenced. For this reason, the vibrational spectroscopy does not help to estimate the contribution of the two final forms – benzenoid and quinoid in the real electronic structure of the dyes. IR and Raman data for the various dyes are with close numerical values to that of the predicted ones.

B.4.4. Hubenova Y., **Todorova M.**, Bakalska R., Mitov M. *Photophysical and Electrochemical Properties of Newly Synthesized Stilbazolium Dyes*. ChemElectroChem **2022**, *9 (24)*, e202200918. **ISSN** 21960216
DOI: 10.1002/celc.202200918
(IF$_{(2022)}$ 4.0, **SJR$_{(2022)}$** 1.024), Scopus (**Q1**) и Web of Science (**Q2**)

In this study, the spectral and electrochemical properties of three newly synthesized stilbazolium dyes possessing an enlarged π-conjugated system were examined. The synthesis of the dyes was carried by Knoevenagel condensation of 1-butyl-4-methylquinolinium bromide as a common acceptor moiety precursor and different donor moiety precursors (2-hydroxy-naphtaldehyde or 4-hydroxy-naphtaldehyde or 4-N, N-dimethylamino naphthaldehyde). The composition of the produced compounds was confirmed by ^{1}H- and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, elemental analysis, and infrared (IR)-spectroscopy. Their properties including the solvatochromism in different solvents were investigated by ultraviolet-visible (UV-Vis) and fluorescence spectroscopy. The electrochemical properties of the dyes' solutions in methanol were explored by cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS). The optical band-gap values as well as the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy levels of the dyes were estimated based on experimental data and compared. A putative mechanism of electrochemical oxidation/reduction of the investigated dyes was also proposed.

B.4.5. Todorova, M., Bakalska, R., Feizi-Dehnayebi, M., Ziarani, G.M., Pencheva, M., Stojnova, K., Milusheva, M., Nedialkov, P., Cherneva, E., Kolev, T., Nikolova, S. *Synthesis, Anti-Inflammatory Activity, and Docking Simulation of a Novel Styryl Quinolinium Derivative*. Applied Sciences **2025**, 15, 284. https://doi.org/10.3390/app15010284
(IF$_{(2023)}$ 2.5, **SJR$_{(2023)}$** 0.508), Scopus (**Q2**), Web of Science (**Q2**)

Stilbenes, like resveratrol, are natural small molecules with several applications in the treatment of chronic illnesses, diabetes, and neurological and cardiovascular conditions.

Considering the molecular structure of stilbenes and the biological activities of resveratrol, we investigated the synthesis and biological activity of a novel styryl quinolinium (SQ) derivative. The SQ was synthesized using the alkylation of lepidine and methyl 4-methylbenzenesulfonate and further Knoevenagel condensation with 2-hydroxy naphthaldehyde. Its structure was determined using NMR, IR, Raman, UV-Vis, and MS. The current trend of research has shifted toward the synthesis of novel SQ as resveratrol's analog. Therefore, its anti-inflammatory and antioxidant activities were evaluated. The compound exerted very good anti-inflammatory effects in preventing albumin denaturation, which were confirmed by ex vivo immunohistochemical studies. Density functional theory (DFT) analyses were conducted on the SQ structure to gain detailed insights into its active sites, energy gap, quantum properties, and electronic behavior. The biological evaluation of the compound was completed, assessing its antioxidant potential using DPPH radical scavenging assay. In addition, molecular docking simulations were utilized to assess the SQ compound's potential inhibitory effect on human serum albumin (HSA).

B.4.6. Bakalska R., **Todorova M.**, Sbirkova H., Shivachev B., Kolev T. *Comparing of the crystal structure and spectroscopic properties of some stilbazolium dyes with enlarged π-conjugated system I. Chromophores with p-dimethylamino group*. Dyes and Pigments **2017**, *136*, 919 - 929.
DOI: 10.1016/j.dyepig.2016.09.034
ISSN 01437208
(IF(2017) 3.767, SJR(2017) 0.819). Scopus (**Q1**), Web of Science (**Q1**)

A comparative study of the structures and spectroscopic properties of two styrylquinolinium dyes: 4-{(E)-2-[4-(dimethylamino)naphthalen-1-yl]ethenyl}-1-methylquinolinium iodide in an anhydrous (Structure I), and monohydrate (Strucuture II) forms and the newly synthesized dye 4-{(E)-2-[4-(dimethylamino)naphthalen-1-yl]ethenyl}-1-pentylquinolinium bromide dihydrate (III) has been conducted by single crystal X-ray diffraction, IR- and Raman spectroscopy, UV-Vis, steady-state and timeresolved fluorescence spectroscopy, 1H and 13C NMR spectroscopy, and TG/DTA analyses. The structures I, II and III belong to a monoclinic system, space groups $P2_1/c$, $P2_1/n$, and C_2/c, respectively. In all of the crystal structures the molecules in the asymmetric unit are in an E configuration and the naphthyl and quinolinium aromatic rings are nearly planar. The angle between the mean planes of the aromatic rings is in the range 1.43-3.65˚. Calculated BLA (Bond Lengths Alternation) parameters are close to the optimal. Solvatochromism in 12 organic solvents has been investigated by using absorption and emission spectroscopy. Reversal in solvatochromic behavior of the absorption spectra has been observed. The data in solutions indicate a significant charge transfer (CT) band shifting up to 84 nm, corresponding to a great molecular first hyperpolarizability values, and very large Stokes shift up to 245 nm. The average lifetime of the fluorescence of the dye III, determined from the measured data by multi-order exponential decay curve fitting as 34.71 ns in benzene and 2.98 ns in DMSO.

B.4.7. Tumbarski Y., **Todorova M.**, Topuzova M., Gineva G., Yanakieva V., Ivanov I., Petkova N. *Comparative Study on Physicochemical, Antioxidant and Antimicrobial Properties of Propolis Collected from Different Regions of Bulgaria*. Journal of Apicultural Science **2023**, *67 (1)*, 37 - 56.
DOI: 10.2478/jas-2023-0004

(IF(2023) 0.7 SJR(2023) 0.508). Scopus (**Q3**), Web of Science (**Q4**)

Propolis (bee glue), a valuable biological mixture produced by European honey bees (Apis mellifera L.), is widely used as a remedy in traditional and alternative medicine, as an effective food biopreservative and as a nutritional value enhancer. The present study aimed to investigate eighty propolis samples collected from all twenty-eight districts of Bulgaria in the period of 2020-2022 in order to determine their physicochemical properties as well as their antioxidant and antimicrobial potential. The raw propolis samples exhibited brown or green colour depending on the climatic zone from which they are collected and pH values from 4.82 to 5.87, while the moisture content ranged between 0.98% and 2.97%. The total phenolics content (TPC) and total flavonoids content (TFC) varied from 63.14 mg GAE/g to 737.27 mg GAE/g, and from 29.22 mg QE/g to 234.17 mg QE/g, respectively. The propolis samples demonstrated significant antioxidant potential determined by DPPH and FRAP methods, from 18.56 mM TE/g to 1598.66 mM TE/g and from 82.28 mM TE/g to 1208.81 mM TE/g, respectively, whose values showed a positive correlation (r^2) with the TPC and TFC. The results from the antimicrobial screening revealed that the methanolic propolis extracts in concentration of 20 mg/ml exhibited the highest inhibitory effect on the Gram-positive bacteria such as *Micrococcus luteus* 2YC-YT, *Bacillus subtilis* ATCC 6633, Staphylococcus aureus ATCC 25923, *Listeria monocytogenes* NBIMCC 8632 and *Listeria innocua* ATCC 33090.

B.4.8. Tumbarski Y., Topuzova M., **Todorova M.** *FOOD INDUSTRY APPLICATIONS OF PROPOLIS: A REVIEW*. Journal of Hygienic Engineering and Design **2022**, 40, 257 - 265. **(SJR(2022) 0.168),** Scopus (**Q4**)

Nowadays, the increasing consumer’s demands for fresh and minimally processed foods without chemical additives attracted the research attention on some novel methods in food industry and use of natural compounds as alternative of chemical preservatives. As a unique natural product obtained from beekeeping, propolis possesses a wide range of biological activities and health benefits that can be used in food production and biopreservation. Propolis (bee glue) is a sticky resinous substance that is collected and processed by honey bees (Apis mellifera L.) from various plant sources such as flowers, leaf buds and tree exudates, and serving as a building and defensive material in their hives. This review discusses the trends in application of propolis as a safe, innovative and promising approach to quality improvement and natural preservation of different food products. The broad antimicrobial spectrum of propolis against spoilage microorganisms and foodborne pathogens offers a great variety of applications in food industry for biopreservation of meat, fish and poultry products, eggs, milk and dairy products, perishable fruits, vegetables, fruit juices and other beverages. In addition to its antimicrobial potential, the strong antioxidant properties of propolis can contribute to increase the nutritional value of the products or to retard the lipid oxidation and protein degradation of processed foods. For this purpose, propolis can be added directly to the food matrix in the form of an extract, to be applied on the surface of the product as a bioactive film or edible coating, or to be included in the composition of food biopackaging materials, thus preventing the food spoilage and enhancing the storage life of the food products. The present study on the applications of propolis in the food industry worldwide and its valuable properties reveals the potential of this natural product as a food additive, as a functional food ingredient, and as a prospective food biopreservative agent prolonging the shelf-life and improving the quality of food products.

B.4.9. Tumbarski Y.D., **Todorova M.M.**, Topuzova M.G., Georgieva P.I., Petkova N.T., Ivanov I.G. *Postharvest Biopreservation of Fresh Blueberries by Propolis-Containing Edible Coatings Under Refrigerated Conditions*. Current Research in Nutrition and Food Science **2022**, *10 (1)*, 99 - 112.
DOI: 10.12944/CRNFSJ.10.1.08
(IF$_{\mathbf{(2023)}}$ 0.9, **SJR$_{\mathbf{(2023)}}$** 0.256), Scopus (**Q3**), Web of Science (**Q4**)

Propolis is a complex honey bee product known for its antioxidant potential and antimicrobial activity, widely used as a food biopreservative and food additive. The object of the current research was to investigate the effects of carboxymethyl cellulose (CMC) edible coatings applied alone and in combination with a propolis extract on the quality parameters and storage life of fresh blueberries during refrigerated storage for 20 days. For this purpose, three experimental groups were prepared: blueberries without coating (control group), blueberries with 1%CMC coatings and blueberries with 1% CMC coatings+1% propolis extract (CMC+P). During the storage, the physicochemical and microbiological parameters of the experimental groups were evaluated. The use of CMC and CMC+P coatings reduced the weight loss by 1.13% and 1.67% in comparison with the control group on the 20-th day of storage. A significant decrease in decay percentage was found, which was in the great extent in the CMC+P coated fruit compared to the CMC coatings and the control fruit. The CMC and CMC+P edible coatings did not affect the TSS levels, the decreasing TA and increasing pH values. The application of CMC and CMC+P coatings did not cause a protective effect on the lowering values of total phenolic and anthocyanin contents in both treatments, but exhibited a positive influence on the antioxidant activity in the coated blueberries. During the entire storage period, propolis containing edible coatings (CMC+P) reduced the bacterial, yeasts and fungal counts, visibly expressed by a reduction in decay incidence in comparison with the uncoated and CMC-treated fruit. Therefore, the application of propolis in the composition of edible coatings can be considered as an effective approach for improving the postharvest quality and prolonging the storage life of fresh blueberries.

B.4.10. Tumbarski Y.D., **Todorova M.M.**, Topuzova M.G., Georgieva P.I., Ganeva Z.A., Mihov R.B., Yanakieva V.B. *Antifungal activity of carboxymethyl cellulose edible films enriched with propolis extracts and their role in improvement of the storage life of kashkaval cheese*. Current Research in Nutrition and Food Science **2021**, *9 (2)*, 487 - 499.
(SJR$_{\mathbf{(2021)}}$ 0.254), Scopus (**Q3**), Web of Science (**Q4**)

Propolis (bee glue) is a complex biological product known for its antimicrobial activity and antioxidant potential, and widely used as food additive and biopreservative. The aim of the present research was to examine the effects of propolis on the improvement of the storage life of ripened kashkaval cheese. For this purpose, three types of 1% carboxymethyl cellulose edible films containing three different ethanolic propolis extracts in concentration of 1% were prepared. Double-layer edible films were applied on the kashkaval cheese surface, and the samples were designated as T1, T2, and T3. An untreated sample was kept as a control. The four experimental groups (T1, T2, T3, and control) were then stored under refrigeration conditions at 4°C and 75% relative humidity (RH) for 56 days. During the storage period, the cheese was observed for visible changes, and samples for physicochemical and microbiological analyses were taken. The results from physicochemical analysis demonstrated that values of titratable acidity decreased in all experiment groups during the storage period. The results from microbiological analysis revealed that three types of edible films T1, T2, and T3 did not affect the decreasing number of lactic acid bacteria, a normal process for the period after the cheese ripening. The application of T1, T2, and T3 edible films did not affect the increasing number of yeasts in kashkaval cheese through the storage period. However, T1 and T2 edible films effectively inhibited the fungal growth on cheese surface, and no signs of spoilage were observed until the end of the storage for 56 days. In contrast, T3 treatment delayed but did not inhibit the fungal growth, and the first signs of fungal decay appeared between the 35th and 42nd day of the storage, compared to the control (28th day). Thus, the application of propolis can be considered an effective means for the prevention of fungal spoilage of kashkaval cheese.

Г.7.1. Gerasimova A., Nikolova K., Petkova N., Ivanov I., Dincheva I., Tumbarski Y., Yanakieva V., **Todorova M.**, Gentscheva G., Gavrilova A., Yotkovska I., Nikolova S., Slavov P., Harbaliev N. Metabolic Profile of Leaves and Pulp of *Passiflora caerulea* L. (Bulgaria) and Their Biological Activities. *Plants* **2024**, *13 (13)*, 1731.
(IF$_{(2023)}$ **4.0**, SJR$_{(2023)}$ **0.795**), Scopus (**Q1**), Web of Science (**Q1**)

At present, there are no data in the scientific literature on studies aimed at characterizing Passiflora caerulea L. growing in Bulgaria. The present study aimed to investigate the metabolic profile and elemental composition of the leaves and pulp of this Passiflora, as well as to evaluate the antioxidant, antimicrobial and anti-inflammatory activities of its leaf and pulp extracts. The results showed that the pulp predominantly contained the essential amino acid histidine (7.81 mg g^{-1}), while it was absent in the leaves, with the highest concentration being tryptophan (8.30 mg g^{-1}). Of the fatty acids, palmitoleic acid predominated both in the pulp and in the leaves. A major sterol component was β-sitosterol. Fructose (7.50%) was the predominant sugar in the pulp, while for the leaves, it was glucose—1.51%. Seven elements were identified: sodium, potassium, iron, magnesium, manganese, copper and zinc. The highest concentrations of K and Mg were in the pulp (23,946 mg kg^{-1} and 1890 mg kg^{-1}) and leaves (36,179 mg kg^{-1} and 5064 mg kg^{-1}). According to the DPPH, FRAP and CUPRAC methods, the highest values for antioxidant activity were found in 70% ethanolic extracts of the leaves, while for the ABTS method, the highest value was found in 50% ethanolic extracts. In the pulp, for all four methods, the highest values were determined at 50% ethanolic extracts. Regarding the antibacterial activity, the 50% ethanolic leaf extracts were more effective against the Gram-positive bacteria. At the same time, the

70% ethanolic leaf extract was more effective against Gram-negative bacteria such as Salmonella enteritidis ATCC 13076. The leaf extracts exhibited higher anti-inflammatory activity than the extracts prepared from the pulp. The obtained results revealed that P. caerulea is a plant that can be successfully applied as an active ingredient in various nutritional supplements or cosmetic products.

Г.7.2. Ivanov I., **Todorova M.**, Petkova N., Dincheva I. Non-polar phytochemical compounds from dandelion (Taraxacum officinale Weber ex F.H. Wigg.) flowers. *Bulgarian Chemical Communications* **2024**, *56*, 96 - 99.
(SJR$_{(2023)}$ **0.148**), Scopus (**Q4**)

Dandelion is a well-known edible and medicinal plant with numerous studies of its health benefits. Interest presents its polyphenolic and carbohydrate composition and its non-polar components in the aerial parts and roots have been identified. There are no studies about the composition of the fatty acid phytocomponents in the flowers of the dandelion. The main purpose of the present study is a comparative investigation of n-hexane, ethanol, and n-hexane/ethanol (1:1 v/v) soluble compounds from dandelion flowers (Taraxacum officinale Weber ex F.H. Wigg.) collected during the flowering period. The GC-MS analysis of the non-polar (lipid) fractions showed the presence of 30 biologically active phytocompounds. The fatty acids predominated in the investigated extracts [(50-60 % of total ion current (TIC)], followed by triterpenes (9-11 % of TIC) and phytosterols (7-8 % of TIC). Polyunsaturated fatty acids - linoleic acid and α-linolenic acid (10 – 15 % of TIC) were identified as the major components. Phytosterols were mainly represented by β-sitosterol (3-4 % of TIC) and stigmasterol (above 2% of TIC), while pentacyclic triterpenes from cycloartenol 3-acetate (3-4% of TIC) and β-amyrin (above 2% of TIC) were found. Based on the fatty acid profile, the nutritional indices directly correlated with the lipid metabolites profile responsible for human health were calculated: Index of atherogenicity (IA) – 1.2-1.6; Index of thrombogenicity (IT) – 0,6-0,7; Hypocholesterolemic/hypercholesterolemic (HH) ratio – 1.0-1.3; Health-promoting index (HPI) – 0.6-0.8; Unsaturation index (UI) – 72 and linoleic acid/α-linolenic acid (LA/ALA) ratio – 1.1.

Г.7.3. Petrova I., Petkova N., Ivanov I., **Todorova M.**, Ognyanov M., Bileva T., Haytova D. Bioactive compounds and antioxidant activity of extracts from edible flowers of punica granatum and citrus aurantium. *Journal of Hygienic Engineering and Design* **2021**, *33*, 120 - 129.
(SJR$_{(2021)}$ **0.158**), Scopus (**Q4**)

Increasing interest in edible flowers referred on their continuously growing consumption. Extracts of bio-active substances by solvents were the most common method for the processed edible flowers. However, most of the reports deal with ethanol or methanol extracts. Information about the bioactive compounds in water and acetone extracts are limited. Moreover, some reports presented values for fresh weight, while bioavaillability of dry extracts and their antioxidant potential were not discussed in details. Therefore, the object of the current study was to evaluate the content of the bioactive compounds and in vitro antioxidant activity of water and acetone extracts obtained from pomegranate (Punica granatum) and orange trees (Citrus aurantium) edible flowers. The commercial available flowers of pomegranate and orange trees were extracted with water and acetone in an ultrasonic bath. The extracts of edible flowers were analyzed for: carbohydrates (sugars and uronic acids), total chlorophylls, total carotenoids, total phenols, total flavonoids, anthocyanidins. Antioxidant activity was evaluated by four reliable methods (2,2-diphe-nyl-1-picrylhydrazyl-DPPH, 2,2'-azino-bis-3-ethyl-benzthiazoline-6-sulphonic acid-ABTS, ferric reducing antioxidant power-FRAP and cupric reducing antioxi-dant capacity assays-CUPRAC). Orange trees (Citrus aurantium) water extract and acetone extracts from pomegranate (Punica granatum) were obtained in the highest yields. Carbohydrates dominated in orange flowers extracts. Sucrose, glucose and fructose were detected in water extracts of edible flowers. Total chlorophylls, carotenoids, flavonoids and anthocyanidins dominated in acetone extracts from pomegranate. However, water extract of pomegranate contained the highest level of total phenolic compounds 230.8 ± 9.5 mg GAE/g extract. Due to the high total phenolic content, the water extracts of pomegranate demonstrated well pronounced antiox-idant activity. The acetone and water extracts from pomegranate (Punica granatum) and orange trees (Citrus aurantium) edible flowers were evaluated as sources of antioxi-dants. The results reveal the potential application of the obtained edible flower extracts as additives in food and cosmetics.

Г.7.4. Tumbarski, Y., Ivanov, I., **Todorova, M.**, Gerasimova, A., Dincheva, I., Makedonski, L., Nikolova, K. Chemical Composition and Biological Activities of St John's Wort (*Hypericum perforatum L.*) Essential Oil from Bulgaria. *Applied Sciences* **2024**, *14*, 11754.
(IF$_{(2023)}$ **2.5**, SJR$_{(2023)}$ **0.508**), Scopus (**Q2**), Web of Science (**Q2**)

Since ancient times, essential oils obtained from various aromatic plants have been utilized as bioactive ingredients in medicines, foods and cosmetics. The present study aimed to investigate the chemical composition and biological activities of St John's Wort (Hypericum perforatum L.) essential oil (SJW EO) from Bulgaria, which is known to possess various biological properties. Gas chromatography and mass spectrometry (GC–MS) analysis, determination of antioxidant activity (by the ABTS method), an antimicrobial activity test and an in vitro anti-inflammatory activity test were performed. The main classes of compounds identified by GC–MS analysis were monoterpenes (43.55%), followed by sesquiterpenes (36.81%) and alkanes (16.92%). The predominant chemical components of SJW EO were α-pinene (27.52%), followed by β-pinene (10.08%), β-caryophyllene (6.77%), germacrene D (6.37%) and caryophyllene oxide (4.48%). The highest antibacterial activity was observed against the Gram-negative bacteria Klebsiella pneumoniae ATCC 13883 (inhibition zone of 12.0 mm) and Pseudomonas aeruginosa ATCC 9027 (inhibition zone of 11.0 mm). SJW EO exhibited significant in vitro anti-inflammatory activity, as the results demonstrated that its anti-inflammatory effect was stronger than those of the conventional anti-inflammatory drugs Prednisolon Cortico and acetylsalicylic acid (Aspirin), which were used as controls (all in concentration of 1 mg/mL). The obtained results demonstrated that Bulgarian SJW EO can be used as an active ingredient in the composition of new products for the pharmaceutical and cosmetic industries.

Г.7.5. Tumbarski Y., Petkova X., **Todorova M.**, Ivanov I., Deseva I., Mihaylova D., Ibrahim S.A. Effects of pectin-based edible coatings containing a bacteriocin of Bacillus methylotrophicus BM47 on the quality and storage life of fresh blackberries. *Italian Journal of Food Science* **2020**, *32 (2)*, 420 - 427.
(IF$_{(2020)}$ **0.875**, SJR$_{(2020)}$ **0.281**), Scopus (**Q3**), Web of Science (**Q4**)

The aim of the current research is to investigate the effects of edible coatings based on celery pectin singly and in combination with a bacteriocin of Bacillus methylotrophicus BM47 on the quality and storage life of fresh blackberries under refrigeration conditions. In this study three experimental groups were prepared: uncoated blackberries as a control, blackberries with 1% pectin coatings and blackberries with 1% pectin coatings+bacteriocin of B. methylotrophicus BM47. During the storage at 4°C and 75% RH for 16 days, the weight loss, decay percentage, total soluble solids (TSS), titratable acidity (TA), pH, organic acids, sugars, total phenolic content, total anthocyanins and antioxidant activity were analyzed. The results showed that the application of pectin and pectin+bacteriocin coatings led to a reduction in weight loss with 6.3% and 6.7% compared to the control fruit on the 16-th day of storage. A decrease in decay percentage was also noticed, which was most pronounced in the pectin+bacteriocin coated fruit compared to the pectin coatings and control. The pectin and pectin+bacteriocin coatings reduced TSS levels with 0.4% and 0.6%, respectively compared to the control on the 16-th day of the storage, but did not affect TA and pH values. The pectin and pectin+bacteriocin coatings had no effect on decreasing total phenolic and anthocyanin contents or the concentration of sugars (glucose and fructose) in both treatments and the control fruit. The pectin and pectin+bacteriocin edible coatings exhibited a protective effect on the ascorbic acid content, maintaining concentrations of 57.5 mg/100 g of fw and 58.8 mg/100 g of fw (day 16), which were close to the initial values. The pectin and pectin-bacteriocin treatments had also a positive impact on antioxidant activity in the coated blackberries. Both edible coatings effectively inhibited its decrease with the prolongation of the storage time and kept antioxidant levels of 231.8 TE/100 g of fw and 232.4 TE/100 g of fw (day 16) that were close to the initial values.

Г.7.6. Vilhelmova-Ilieva N.M., Nikolova I.N., Nikolova N.Y., Petrova Z.D., Trepechova M.S., Holechek D.I., **Todorova M.M.**, Topuzova M.G., Ivanov I.G., Tumbarski Y.D. *Antiviral Potential of Specially Selected Bulgarian Propolis Extracts: In Vitro Activity against Structurally Different Viruses*. Life **2023**, *13 (7)*, art. no. 1611.
(IF$_{(2023)}$ **3.2**, SJR$_{(2023)}$ **0.713**), Scopus (**Q2**), Web of Science (**Q1**)

Propolis is a natural mixture of resins, wax, and pollen from plant buds and flowers, enriched with enzymes and bee saliva. It also contains various essential oils, vitamins, mineral salts, trace elements, hormones, and ferments. It has been found that propolis possesses antimicrobial, antiviral, and anti-inflammatory properties. We have studied the antiviral activity of six extracts of Bulgarian propolis collected from six districts of Bulgaria. The study was conducted against structurally different viruses: human coronavirus strain OC-43 (HCoV OC-43) and human respiratory syncytial virus type 2 (HRSV-2) (enveloped RNA viruses), human herpes simplex virus type 1 (HSV-1) (enveloped DNA virus), human rhinovirus type 14 (HRV-14) (non-enveloped RNA virus) and human adenovirus type 5 (HadV-5) (non-enveloped DNA virus). The influence of the extracts on the internal replicative cycle of viruses was determined using the cytopathic effect (CPE) inhibition test. The virucidal activity, its impact on the stage of viral adsorption to the host cell, and its protective effect on healthy cells were evaluated using the final dilution method, making them the focal points of interest. The change in viral infectivity under the action of propolis extracts was compared with untreated controls, and Δlgs were determined. Most propolis samples administered during the viral replicative cycle demonstrated the strongest activity against HCoV OC-43 replication. The influence of propolis extracts on the viability of extracellular virions was expressed to a different degree in the various viruses studied, and the effect was significantly stronger in those with an envelope. Almost all extracts significantly inhibited the adsorption step of the herpes virus and, to a less extent, of the coronavirus to the host cell, and some of them applied before viral infection demonstrated a protective effect on healthy cells. Our results enlarge the knowledge about the action of propolis and could open new perspectives for its application in viral infection treatment.

Г.7.7. Petkova N.Tr., Arabadzhiva R.D., Tumbarski Y.D., Todorova M.M., Hambarlyiska I.P., Ivanov I.G., Ibryamova S.F., Ignatova-Ivanova T.V. Physicochemical properties and antimicrobial activity of acetylated chicory fructooligosaccharides. *Philippine Journal of Science* **2021**, *150 (3)*, 995 - 1004.
(IF$_{(2021)}$ **0.85**, SJR$_{(2021)}$ **0.200**), Scopus (**Q3**)

Inulin acetates attract attention as the novel drug carrier. However, the acetylated derivaties of fructooligosaccharides (FOSs) (a low molecular fraction of inulin) were not evaluated. The study aimed to obtain FOSs acetyl esters and to evaluate their foaming properties, water-and oil-holding capacities (WHC and OHC), as well as their antimicrobial activity. One-pot acetylation of chicory FOS with two different degrees of polymerization (DP = 7–9 and 9–12) was performed. The resulting FOSs esters presented white, bitter, water-insoluble substances. The spectroscopic techniques as ultraviolet (UV), Fourier-transform infrared (FTIR), and nuclear magnetic resonance (NMR) spectroscopy were used for characterization and structural elucidation. The antimicrobial activity of acetylated FOSs (1 mg/mL) was tested against 16 microorganisms (Gram-positive and negative bacteria, yeasts, and fungi). Foams prepared with 0.2% FOSs acetates demonstrated the formation of highly stable foams (50–70%). FOSs acetates showed antifungal activity against Fusarium oxysporum and Aspergillus niger and inhibited the growth of yeasts Candida albicans 8673. The inhibition against Gram-positive (Bacillus subtilis 46/H1 and Bacillus subtilis ATCC 6633) and negative (Salmonella abony and Escherichia coli ATCC 8739) bacteria were not observed. However, FOSs acetate with DP 7–9 were active against E. coli 3398, Salmonella typhy 745, and Staphylococus aureus 745 against – which other acetates with DP = 9–12 were inactive. These results demonstrate the potential applications of FOS acetates as a foaming agent and an antifungal substance in pharmaceutical and cosmetic preparations.

Г.7.8. Arabadzhieva R., Ivanov I., Petkova N., **Todorova M.**, Tumbarski Y., Vlaeva I., Vilhelmova-Ilieva N., Nikolova I., Simeonova L. Microwave-assisted synthesis of lactose acetates with antimicrobial, cytotoxic, and antiviral properties. *Biointerface Research in Applied Chemistry* **2022**, *12 (6)*, 8453 - 8470.
(IF$_{(2022)}$ **2.74**, SJR$_{(2022)}$ **0.336**), Scopus (**Q3**), Web of science

The task of this study was to perform the "green" synthesis of lactose octaacetate through microwave irradiation and to establish their biological activities. Lactose ester was prepared after microwave-assisted esterification of lactose with acetic anhydride (yield 85-90%). Lactose octaacetate was characterized by a high degree of acetylation (DS 3.2-3.7). The lactose esters' structure was elucidated by infrared spectroscopy and nuclear magnetic resonance spectroscopy. Lactose octaacetate showed better antifungal activities than antibacterial activities. It possessed slight to moderate antifungal activities against Aspergillus niger ATCC 1015, Penicillium sp., Rhizopus sp., Fusarium moniliforme ATCC 38932. Lactose acetates demonstrated low cytotoxicity against three cell lines: Madin-Darby bovine kidney (MDBK) cells, human epithelial type 2 (HEp-2), and Madin-Darby canine kidney (MDCK) cells. This is the first report for antiviral activity of lactose acetates against herpes simplex virus type 1 (HSV-1), influenza virus A/Panama/2007/99/H3N2 - (IAV/H3N2), PV-1 and Coxsackievirus B1. It was found that this compound showed activity with SI = 2.4 only against PV-1, but against HSV-1, IAV/H3N2, and Coxsackievirus B1 was inactive. The current study demonstrated the applications of lactose acetates as antimicrobial and antiviral substances in food, pharmaceutical, agricultural and cosmetic preparations.

Г.7.9. Milusheva M., Gledacheva V., Stefanova I., Pencheva M., Mihaylova R., Tumbarski Y., Nedialkov P., Cherneva E., **Todorova M.**, Nikolova S. *In Silico, In Vitro, and Ex Vivo Biological Activity of Some Novel Mebeverine Precursors*. Biomedicines **2023**, *11 (2)*, art. no. 605.
(IF$_{(2023)}$ **3.9**, SJR$_{(2023)}$ **0.962**), Scopus (**Q1**), Web of science (**Q2**)

Irritable bowel syndrome (IBS) is a functional gastroenterological disorder with complex pathogenesis and multifaceted therapy approaches, aimed at alleviating clinical symptoms and improving the life quality of patients. Its treatment includes dietary changes and drugs from various pharmacological groups such as antidiarrheals, anticholinergics, serotonin receptor antagonists, targeting chloride ion channels, etc. The present article is focused on the synthesis and biological evaluation of some mebeverine precursors as potential antispasmodics. Methods: In silico analysis aimed at predicting the pharmacodynamic profile of the compounds was performed. Based on these predictions, ex vivo bioelectrical activity (BEA) and immunohistochemical effects of the compounds were established. A thorough biological evaluation of the compounds was conducted assessing their in vitro antimicrobial and cytotoxic activity. Results: All the newly synthesized compounds exerted drug-like properties, whereby 3-methyl-1-phenylbutan-2-amine 3 showed a significant change in BEA due to Ca2+ channel regulation, Ca2+ influx modulation, and a subsequent change in smooth muscle cell response. The immunohistochemical studies showed a good correlation with the obtained data on the BEA, defining amine 3 as a leader structure. No cytotoxicity to human malignant leukemic cell lines (LAMA-84, K-562) was observed for all tested compounds. Conclusion: Based on the experimental results, we outlined 3-methyl-1-phenylbutan-2-amine 3 as a potential effective choice for orally active long-term therapy of IBS.

Г.7.10. 6. Nikolova S., Milusheva M., Gledacheva V., Feizi-Dehnayebi M., Kaynarova L., Georgieva D., Delchev V., Stefanova I., Tumbarski Y., Mihaylova R., Cherneva E., Stoencheva S., **Todorova M.** *Drug-Delivery Silver Nanoparticles: A New Perspective for Phenindione as an Anticoagulant*. Biomedicines **2023**, *11 (8)*, 2201.
(IF$_{(2023)}$ **3.9**, SJR$_{(2023)}$ **0.962**), Scopus (**Q1**), Web of science (**Q2**)

Anticoagulants prevent the blood from developing the coagulation process, which is the primary cause of death in thromboembolic illnesses. Phenindione (PID) is a well-known anticoagulant that is rarely employed because it totally prevents coagulation, which can be a life-threatening complication. The goal of the current study is to synthesize drug-loaded Ag NPs to slow down the coagulation process. Methods: A rapid synthesis and stabilization of silver nanoparticles as drug-delivery systems for phenindione (PID) were applied for the first time. Results: Several methods are used to determine the size of the resulting Ag NPs. Additionally, the drug-release capabilities of Ag NPs were established. Density functional theory (DFT) calculations were performed for the first time to indicate the nature of the interaction between PID and nanostructures. DFT findings supported that galactose-loaded nanostructure could be a proper delivery system for phenindione. The drug-loaded Ag NPs were characterized in vitro for their antimicrobial, cytotoxic, and anticoagulant activities, and ex vivo for spasmolytic activity. The obtained data confirmed the drug-release experiments. Drug-loaded Ag NPs showed that prothrombin time (PT, sec) and activated partial thromboplastin time (APTT, sec) are approximately 1.5 times longer than the normal values, while PID itself stopped coagulation at all. This can make the PID-loaded Ag NPs better therapeutic anticoagulants. PID was compared to PID-loaded Ag NPs in antimicrobial, spasmolytic activity, and cytotoxicity. All the experiments confirmed the drug-release results.

Г.7.11. Milusheva M., Gledacheva V., Stefanova I., Feizi-Dehnayebi M., Mihaylova R., Nedialkov P., Cherneva E., Tumbarski Y., Tsoneva S., **Todorova M.**, Nikolova S. *Synthesis, Molecular Docking, and Biological Evaluation of Novel Anthranilic Acid Hybrid and Its Diamides as Antispasmodics*. International Journal of Molecular Sciences **2023**, *24 (18)*, 13855.
(IF$_{(2023)}$ **4.9**, SJR$_{(2023)}$ **1.179**), Scopus (**Q1**), Web of science (**Q2**)

The present article focuses on the synthesis and biological evaluation of a novel anthranilic acid hybrid and its diamides as antispasmodics. Methods: Due to the predicted in silico methods spasmolytic activity, we synthesized a hybrid molecule of anthranilic acid and 2-(3-chlorophenyl)ethylamine. The obtained hybrid was then applied in acylation with different acyl chlorides. Using in silico analysis, pharmacodynamic profiles of the compounds were predicted. A thorough biological evaluation of the compounds was conducted assessing their in vitro antimicrobial, cytotoxic, anti-inflammatory activity, and ex vivo spasmolytic activity. Density functional theory (DFT) calculation, including geometry optimization, molecular electrostatic potential (MEP) surface, and HOMO-LUMO analysis for the synthesized compounds was conducted using the B3LYP/6–311G(d,p) method to explore the electronic behavior, reactive regions, and stability and chemical reactivity of the compounds. Furthermore, molecular docking simulation along with viscosity measurement indicated that the newly synthesized compounds interact with DNA via groove binding mode. The obtained results from all the experiments demonstrate that the hybrid molecule and its diamides inherit spasmolytic, antimicrobial, and anti-inflammatory capabilities, making them excellent candidates for future medications.

Г.7.12. 4. Milusheva M., **Todorova M.**, Gledacheva V., Stefanova I., Feizi-Dehnayebi M., Pencheva M., Nedialkov P., Tumbarski Y., Yanakieva V., Tsoneva S., Nikolova S. Novel Anthranilic Acid Hybrids—An Alternative Weapon against Inflammatory Diseases. Pharmaceuticals **2023**, *16 (12)*, 1660. (IF$_{(2023)}$ **4.3**, SJR$_{(2023)}$ **0.845**), Scopus (**Q1**), Web of science (**Q1**)

Anti-inflammatory drugs are used to relieve pain, fever, and inflammation while protecting the cardiovascular system. However, the side effects of currently available medications have limited their usage. Due to these adverse effects, there is a significant need for new drugs. The current trend of research has shifted towards the synthesis of novel anthranilic acid hybrids as anti-inflammatory agents. Phenyl- or benzyl-substituted hybrids exerted very good anti-inflammatory effects in preventing albumin denaturation. To confirm their anti-inflammatory effects, additional ex vivo tests were conducted. These immunohistochemical studies explicated the same compounds with better anti-inflammatory potential. To determine the binding affinity and interaction mode, as well as to explain the anti-inflammatory activities, the molecular docking simulation of the compounds was investigated against human serum albumin. The biological evaluation of the compounds was completed, assessing their antimicrobial activity and spasmolytic effect. Based on the experimental data, we can conclude that a collection of novel hybrids was successfully synthesized, and they can be considered anti-inflammatory drug candidates—alternatives to current therapeutics.

Г.7.13. Todorova M., Milusheva M., Kaynarova L., Georgieva D., Delchev V., Simeonova S., Pilicheva B., Nikolova S. *Drug-Loaded Silver Nanoparticles—A Tool for Delivery of a Mebeverine Precursor in Inflammatory Bowel Diseases Treatment*. Biomedicines **2023**, *11 (6)*, 1593.
(IF$_{(2023)}$ **3.9**, SJR$_{(2023)}$ **0.962**), Scopus (**Q1**), Web of science (**Q2**)

Chronic, multifactorial illnesses of the gastrointestinal tract include inflammatory bowel diseases. One of the greatest methods for regulated medicine administration in a particular region of inflammation is the nanoparticle system. Silver nanoparticles (Ag NPs) have been utilized as drug delivery systems in the pharmaceutical industry. The goal of the current study is to synthesize drug-loaded Ag NPs using a previously described 3-methyl-1-phenylbutan-2-amine, as a mebeverine precursor (MP). Methods: A green, galactose-assisted method for the rapid synthesis and stabilization of Ag NPs as a drug-delivery system is presented. Galactose was used as a reducing and capping agent forming a thin layer encasing the nanoparticles. Results: The structure, size distribution, zeta potential, surface charge, and the role of the capping agent of drug-loaded Ag NPs were discussed. The drug release of the MP-loaded Ag NPs was also investigated. The Ag NPs indicated a very good drug release between 80 and 85%. Based on the preliminary results, Ag NPs might be a promising medication delivery system for MP and a useful treatment option for inflammatory bowel disease. Therefore, future research into the potential medical applications of the produced Ag NPs is necessary.

Г.7.14. Stoyanova M., Milusheva M., Gledacheva V., Stefanova I., **Todorova M.**, Kircheva N., Angelova S., Pencheva M., Stojnova K., Tsoneva S., Nikolova S. *Spasmolytic Activity and Anti-Inflammatory Effect of Novel Mebeverine Derivatives* Biomedicines **2024**, *12 (10)*, 2321.
(IF$_{(2023)}$ **3.9**, SJR$_{(2023)}$ **0.962**), Scopus (**Q1**), Web of science (**Q2**)

Background: Irritable bowel syndrome (IBS) has a major negative influence on quality of life, causing cramps, stomach pain, bloating, constipation, etc. Antispasmodics have varying degrees of efficacy. Mebeverine, for example, works by controlling bowel movements and relaxing the muscles of the intestines but has side effects. Therefore, more efficient medication is required. Methods: In the current study, we investigated the synthesis of novel mebeverine analogs and determined ex vivo their spasmolytic and in vitro and ex vivo anti-inflammatory properties. The ability to influence both contractility and inflammation provides a dual-action approach, offering a comprehensive solution for the prevention and treatment of both conditions. Results: The results showed that all the compounds have better spasmolytic activity than mebeverine and good anti-inflammatory potential. Among the tested compounds, 3, 4a, and 4b have been pointed out as the most active in all the studies conducted. To understand their mechanism of activity, molecular docking simulation was investigated. The docking analysis explained the biological activities with their calculated Gibbs energies and possibilities for binding both centers of albumin. Moreover, the calculations showed that molecules can bind also the two muscarinic receptors and interleukin-β, hence these structures would exert a positive therapeutic effect owed to interaction with these specific receptors/cytokine. Conclusions: Three of the tested compounds have emerged as the most active and effective in all the studies conducted. Future in vivo and preclinical experiments will contribute to the establishment of these novel mebeverine derivatives as potential drug candidates against inflammatory diseases in the gastrointestinal tract.

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