Chemia Naissensis, Vol 7, Issue 2, 23-34, SHORT COMMUNICATION,
https://doi.org/10.46793/ChemN7.2.23K

Density Functional Theory (DFT) Investigation on the Radical-Scavenging Activity of Galantamine and Norgalantamine

Running title: Computational studies on galantamine and norgalantamine

Veronika Karadjova[1], Luciano Saso[2], Biljana Arsić[3], Zhivko Velkov[4*]

1-University of Chemical Technology and Metallurgy, 8 Kliment Ohridski blvd., 1756 Sofia, Bulgaria

2-Sapienza University of Rome, Department of Physiology and Pharmacology, Rome, Italy

3-University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Republic of Serbia

4- Department of Chemistry, South-West University "Neofit Rilski", 2700-Blagoevgrad, 66 Ivan Mihajlov Str, Bulgaria

Veronika Karadjova: vkar@mail.bg
Luciano Saso: luciano.saso@uniroma1.it, ORCID: https://orcid.org/0000-0003-4530-8706
Biljana Arsić: biljana.arsic@pmf.edu.rs, ORCID: https://orcid.org/0000-0002-1248-5864
Zhivko Velkov: jivko_av@abv.bg, ORCID: https://orcid.org/0000-0003-4097-1250

Chemia Naissensis, Vol 7, Issue 2, 23-34, SHORT COMMUNICATION,
https://doi.org/10.46793/ChemN7.2.23K

ABSTRACT

Galantamine, a natural alkaloid with inherent antioxidant properties, effectively crosses the blood-brain barrier, making it a promising therapeutic agent for treating certain brain-related disorders in humans. This density functional theory (DFT) study presents the results of quantum chemical calculations on the dissociation enthalpies of galantamine's O-H and C-H bonds, elucidating its radical-scavenging activities. The findings highlight galantamine's propensity to interact with radicals in biological systems, emphasizing the bond strength and acidity of its O-H and C-H groups. Additionally, the study explores the implications of hydride ion abstraction, shedding light on its potential reactivity and antioxidant mechanisms.

Keywords: *galantamine, norgalantamine, radical-scavenging activity, DFT calculations*

Introduction

Oxidative damage is a critical factor in the development of many diseases. The brain is especially vulnerable due to its high content of easily oxidizable fatty acids, significant oxygen consumption, and limited endogenous antioxidant defences (Behl & Moosman, 2002). Within this context, oxidative stress emerges as a pivotal player in the neuronal degeneration observed in the brains of Alzheimer's disease patients (Ansari & Scheff, 2010; Marlatt et al., 2008; Petersen et al., 2007). Reactive oxygen species (ROS) instigate cell membrane dysfunction (Axelsen et al., 2011), subsequently triggering the initiation of apoptosis in nerve cells (Behl & Moosman, 2002).

Plant antioxidants with radical-scavenging activity have the potential to protect against brain diseases or, at the very least, slow their progression. However, their efficacy largely depends on their ability to cross the blood-brain barrier. Galantamine, renowned for its radical-scavenging properties (Traykova et al., 2003), efficiently crosses the blood-brain barrier while exhibiting relatively low toxicity. This makes it a reliable therapeutic option for treating various brain disorders in humans (Tsvetkova et al., 2013).

The antioxidant properties of galantamine hydrobromide have been substantiated through *in vitro* experiments (Traykova et al., 2003). The capacity of galantamine and galantamine hydrobromide to neutralize ROS such as $O_2^{\bullet-}$, $^{\bullet}OH$, and HOCl is attributed to the hydroxyl group at the sixth position of the galantamine cyclohexene ring (Figure 1). This conclusion is based on the observation that any modification of the hydroxyl group affects the efficacy of radical-scavenging activity (RSA) (Karadjova et al., 2021). Its effectiveness disappears entirely upon the alkylation of the OH group. Moreover, research indicates an increase in galantamine's radical-scavenging potency when it is converted into its hydrobromide salt form.

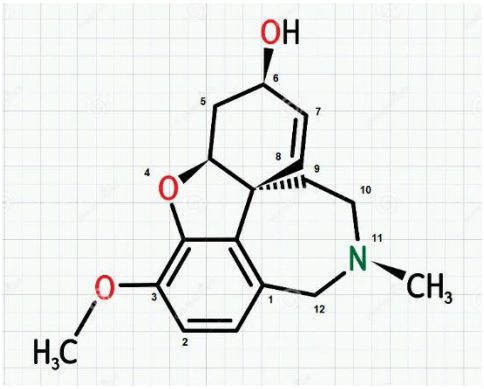


Figure 1. Structure of galantamine.

Galantamine has a significant impact on a variety of human brain pathologies (Harvey, 1995; Marco & do Carmo Carreiras, 2006; Melo et al., 2009). Its primary application is as a competitive, reversible, and selective inhibitor of acetylcholinesterase, which enhances cognitive function in

individuals with Alzheimer's disease. Additionally, it has been observed to play a role in nicotine addiction (Harvey, 1995).

The quantum chemical studies available in literature primarily focus on the electron density distribution and the resulting electrostatic potential of galantamine (Parameswari et al., 2013). Alternatively, some studies delve into the correlation between the energy of the highest occupied orbitals and their role as inhibitors of acetylcholinesterase within a group of galantamine analogues (Ece & Pejin, 2015). Yet, none of these studies have explored galantamine's propensity for engaging in chemical reactions linked to its most readily dissociable O-H and C-H bonds.

In this paper we will discuss the results of quantum chemical calculations of the enthalpies of dissociation of O-H and C-H bonds in galantamine (Figure 1 and the Schemes 1-5) and norgalantamine - an analogue of galantamine without the methyl group at N11. Norgalantamine is widely used in synthetic chemistry (Vezenkov et al., 2009, 2020). The results provide valuable insights into their overall reactivity, the mechanism of radical scavenging activity, and the most likely metabolic pathways.

Experimental

The calculations were performed using the density functional theory and the hybrid Becke-3-parameter-Lee-Yang-Parrfunctional (DFT/B3LYP) coded in the Gaussian09 software package (Frisch et al., 2016) and orbital basis 6-311++g(d,p) (Parr, 1980). The aforementioned procedure was previously employed on a different set of radical-scavengers, yielding entirely satisfactory outcomes (Velkov et al., 2019). No constraints on geometry were imposed during the optimization. The energy minima for all structures were confirmed by frequency analysis. Solvent effects were calculated with the self-consistent reaction field (SCRF) approach of the polarized continuum model (PCM) (Tomasi et al., 2005).

The species (X) enthalpy is calculated using the following equation:

$$H(X) = E_0 + ZPE + \Delta H_{trans} + \Delta H_{rot} + \Delta H_{vib} + RT$$

where E_0 is the total electronic energy, ZPE is the unscaled zero-point energy, ΔH_{trans}, ΔH_{rot}, and ΔH_{vib} are respectively the translational, rotational, and vibrational shares in the enthalpy, RT represents the work term converting the internal energy into enthalpy (T = 298 K).

The enthalpies of the hydrogen atom, proton and electron in vacuum and in water are taken from the literature (Markovic et al., 2016; Rimarcik et al., 2010). The used proton enthalpy ($\mathbf{H}_{(H^+)}$) in vacuum is 6.197 kJ/mol, and in water is –1083.803 kJ/mol; the used enthalpy of an electron ($\mathbf{H}_{(e^-)}$) in vacuum is 3.145.kJ/mol, and in water is –232.676 kJ/mol, the used enthalpy of a hydrogen atom ($\mathbf{H}_{(H^\cdot)}$) in vacuum is –1312.479 kJ/mol and in water is –1316.479 kJ/mol.

Results and Discussion

Table 1 provides the obtained enthalpies of O-H and C-H bond dissociations.

Table 1. Enthalpies of O-H and C-H bond dissociations (in kJ/mol, in water).

	BDE	IP	PDE	PA	ETE
C-H galantamine	321.81	288.16	24.46	316.00	-3.38
C-H norgalantamine	321.80	308.33	4.28	316.52	-3.61
O-H galantamine	428.52	288.16	131.16	218.26	201.07
O-H norgalantamine	428.44	308.33	110.92	218.18	201.07

X-H bond breaking. HAT mechanism of O-H bond dissociation

Scheme 1. HAT mechanism

The most prevalent mechanism for X–H bond dissociation in a nonpolar environment is the Hydrogen Atom Transfer (HAT). The characteristic descriptor of this mechanism is the bond dissociation enthalpy (BDE), which is calculated as the enthalpy difference between the resultant radical and hydrogen atom and the corresponding galantamine molecule: BDE = H(Gal-O•) + H(H•) - H(Gal-OH). The spin density within the radical, which emerges subsequent to the homolytic dissociation of the hydroxyl group, is notably centred at the oxygen atom. The Mulliken spin density at this oxygen is substantial – 0.85. The calculated O-H BDE confirmed that a dissociation of this bond by the HAT mechanism is quite difficult - BDE is 428.52 kJ/mol.

In tandem with galantamine, we also computed the O-H bond BDE for norgalantamine. The only structural difference between the two compounds is the presence of a methyl group linked to the nitrogen at position 11 (Figure 1). However, this distinction does not exert any influence on the BDE of the O-H bond at position 6. In norgalantamine it is 428.44 kJ/mol. For the sake of comparison, it's worth noting that the calculated BDEs for the most reactive hydroxyl groups in flavones and coumarins fall within the range of 320 to 350 kJ/mol (Karadjova et al., 2021).

The results in Table 1 are calculated taking into account the polarizing effect of water. Since the HAT mechanism is expected to take place in non-polar environments, such as those in the human brain, we calculated the BDE of the O-H bond in a vacuum (Table 2). It is not surprising that,

within a vacuum, these bonds exhibit a greater propensity for dissociation compared to their behavior in a water environment (Table 1).

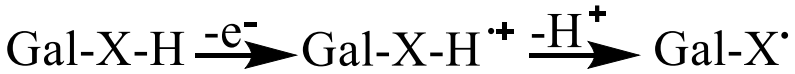
Table 2. BDE in a vacuum (in kJ/mol)

	Galantamine	Norgalantamine
BDE(O-H)	425.53	425.27
BDE(C-H)	319.78	319.65

Therefore, the radical-scavenging reaction involving the hydroxyl group through the HAT mechanism appears to be unattainable due to thermodynamic constraints. However, it gains greater feasibility within nonpolar environments.

SET-PT mechanism of O-H bond dissociation

Two-steps mechanisms for O-H bond dissociation exhibit higher likelihood within polar environments. Within this mechanism, an initial step involves the detachment of an electron from galantamine (or norgalantamine), followed by the subsequent removal of a proton from the resulting cation-radical (Scheme 2).



Scheme 2. SET-PT mechanism

The feasibility of the SET-PT mechanism can be approximated through the assessment of the ionization potential ($IP = H_{(Gal\text{-}O\text{-}H^{\bullet+})} + H_{(e-)} - H_{(Gal\text{-}O\text{-}H)}$) and the proton dissociation enthalpy ($PDE = H_{(Gal\text{-}X^{\bullet})} + H_{(H+)} - H_{(Gal\text{-}X\text{-}O^{\bullet+})}$).

The process of electron detachment from galantamine (in water) is more likely to occur compared to homolytic dissociation (in vacuum). IP of galantamine (288.16 kJ/mol) (norgalantamine 308.33 kJ/mol) is less than BDE, even in vacuum (319.78 kJ/mol).

Proton separation from the O-H group in the cation-radical of galantamine (and norgalantamine) in the second step of the SET-PT mechanism is even easier: the change in the enthalpy of this process (PDE) is 131.16 kJ/mol (110.92 kJ/mol for norgalantamine).

Therefore, the dissociation of the O-H bond in galantamine and the norgalantamine by the SET-PT mechanism is more probable than the dissociation by the HAT mechanism. The ionization

potential of the phenolic compounds we have worked with before is between 369 and up to 393 kJ/mol (Markovic et al., 2016), and the PDE is a small positive or even negative number.

SPLET mechanism of O-H bond dissociation

Scheme 3. SPLET mechanism

For the description of the SPLET (Sequential Proton Loss Electron Transfer) mechanism (Scheme 3) the proton affinity {PA = H(Gal-O$^-$) + H(H$^+$) - H(Gal-OH)} and the electron transfer enthalpy ETE = H(Gal-O$^\bullet$) + H(e$^-$) - H(Gal-O$^-$) are needed.

The enthalpy of heterolytic dissociation of the O-H bond (PA) in galantamine is 218.26 kJ/mol (in norgalantamine, 218.18 kJ/mol). Simultaneously, the enthalpy of electron transfer (ETE) for both compounds is 201.07 kJ/mol. In this case, the first stage of the SPLET mechanism – the heterolytic dissociation of the O-H bond presents greater difficulty. However, the results indicate that SPLET is the most likely mechanism for O-H bond dissociation.

These results strongly suggest that O-H bond within galantamine plays a pivotal role in defining its chemical and physiological properties.

Protonated galantamine and norgalantamine

To verify the experimentally found results (Traykova et al., 2003), which indicate that the RSA of galantamine increases when it is converted to the hydrobromide salt, we calculated the PA and ETE of the O-H bonds in protonated galantamine and norgalantamine. It turns out that the PA of the O-H bond in the protonated galantamine is lower: 210.68 kJ/mol (respectively, 210.39 kJ/mol for the norgalantamine), and the ETE for the detachment of an electron from the obtained anion from the hydroxyl group is 209.34 kJ/mol (209.62 kJ/mol for norgalantamine) (Table 3).

Table 3. Enthalpies of C-H и O-H bond dissociation (kJ/mol) in the protonated galantamine and norgalantamine in the SPLET mechanism.

	PA_{O-H}	ETE_{O-H}	PA_{C-H}	$ETE_{C-anion}$
Galantamine cation	210.68	209.34	295.83	-14.67
Norgalantamine cation	210.39	209.62	295.87	-14.63

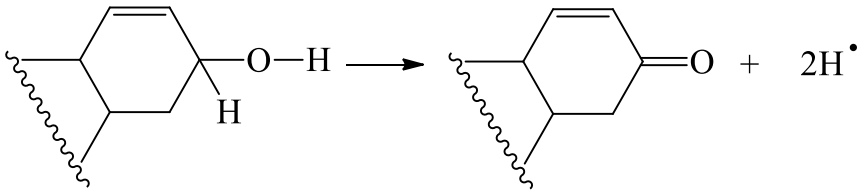
PA in galantamine (and norgalantamine) with the protonated amino is about 8 kJ/mol less than that in the non-protonated, which agrees with the experimental results, according to which in an acidic environment galantamine is a more active radical-scavenger.

The O-H bond in protonated galantamine (and norgalantamine) dissociates more easily *via* the SPLET mechanism compared to the unprotonated form, which accounts for the higher RSA observed in its bromide salt.

On the other hand, this supports the conclusion that the SPLET mechanism occurs during galantamine's reaction with active radicals *in vitro*.

Dissociation of the C(6)-H bond

The C-H bond located at the C(6) position (Scheme 1) is anticipated to undergo relatively easier dissociation across all mechanisms. This is attributed to the proximity of the double bond and the oxygen atom. The spatial arrangement facilitates the sharing of spin density and charge with neighboring carbon atoms (C7 and C8) as well as the oxygen, if a positive charge is localized at the C(6) position. Certainly, the galantamine radical, acquired after the homolytic dissociation (Scheme 4) of the C-H bond, manifests a maximum spin density of 0.676. This is significantly less than in the radical obtained after the homolytic dissociation of the O-H bond. BDE of this C-H bond in galantamine (in water) is 321.81 kJ/mol, (and 321.80 kJ/mol in norgalantamine). This is about 107 kJ/mol lower than the BDE of the O-H bond (Table 1). As mentioned above, it is necessary to inspect the HAT mechanism for C-H bond dissociation in a vacuum (Table 2). It turned out that BDE of this bond in galantamine is 319.78 kJ/mol, and in norgalantamine: 319.65 kJ/mol.



Scheme 4. Conversion to oxygalantamine.

If a HAT mechanism of the C-H bond dissociation is realized, it becomes possible at the next step to break much easier the O-H bond according to Scheme 4, and the enthalpy change of the O-H bond becomes now 148.82 kJ/mol (148.60 kJ/mol for norgalantamine).

Let us consider the changes in the enthalpies in the two-steps mechanisms of C-H bond breaking. The conversion of galantamine to a cation-radical in the SET-PT mechanism has already been considered (Scheme 2). As said above, this is a completely feasible process from a thermodynamic point of view: IP is 288.16 kJ/mol (308.33 kJ/mol for norgalantamine). PDE of the C-H bond

dissociation from the cation-radical of galantamine is 24.457 kJ/mol (4.280 kJ/mol in norgalantamine). It turns out that it is much easier to tear off a proton from the C-H bond than from the O-H bond of the cation-radical.

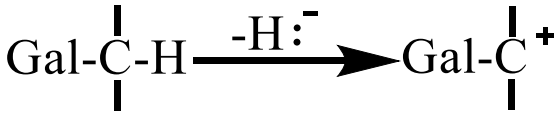
Heterolytic dissociation of the C-H bond to an anion and a proton is less possible than the dissociation of the O-H bond. PA of the C-H bond dissociation is 316.00 kJ/mol in galantamine (316.52 kJ/mol in norgalantamine). If such an anion is formed, it would spontaneously release an electron to form a radical (the ETE of the galantamine anion is -3.38 kJ/mol and of the norgalantamine anion -3.61 kJ/mol).

The protonation of the amino group in galantamine and norgalantamine may benefit the SPLET mechanism (Table 3). The enthalpy of the C-H bond heterolytic dissociation (PA) in the protonated galantamine is 295.83 kJ/mol (in the protonated norgalantamine: 295.87 kJ/mol). ETE of the anions with a protonated amino group are bigger negative numbers: -14.67 kJ/mol (and -14.63 kJ/mol).

In a non-polar environment, galantamine readily undergoes oxidation to oxygalantamine during its interaction with two active radicals.

Hydride ion detachment

The presence of a heteroatom and a conjugate system adjacent to C(6) and C(12) are the reasons to consider another way of C-H bond dissociation - the detachment of the hydride ion according to Scheme 5:



Scheme 5. Hydride ion separation

A positive charge in these positions can be readily delocalized due to the nearby double bond and heteroatom.

The change in the enthalpy of this process at C6 is 319.72 kJ/mol (in norgalantamine: 318.48 kJ/mol). The same dissociation at C12 adjacent to the nitrogen atom, is more probable: 228.51 kJ/mol for galantamine and 237.69 kJ/mol for norgalantamine. The probability of this process is therefore fully comparable to the SPLET dissociation mechanism of the O-H bond.

For comparison, we calculated the change in enthalpy during the detachment of the hydride ion from NADH (202.62 kJ/mol), and from morphine, where the change in enthalpy is 187.72 kJ/mol.

Conclusion

Using DFT methods, the dissociation enthalpies of the O-H bond and the two most easily dissociable C-H bonds in galantamine and norgalantamine were calculated. The calculations were performed in a non-polar environment (vacuum) and with consideration of the polarizing effect of water. This approach enables modeling the RSA of these compounds in aqueous and lipid environments within the human body. To explain the experimentally observed higher activity of galantamine hydrobromide, studies were conducted on both the free bases and the protonated forms of the two alkaloids. It was found that the higher activity of the salt is due to the preferred SPLET mechanism and the greater acidity of the O-H bond in the ammonium ion compared to its acidity in the free base. The possibility of two-electron oxidation processes was also evaluated: a two-step oxidation of galantamine *via* the HAT mechanism to oxygalantamine and a one-step process involving hydride ion abstraction from the C12 atom, located adjacent to the aromatic ring A and the nitrogen atom.

Conflict-of-Interest Statement

The authors declare that they have no conflict of interest.

References

Ansari, M. A., & Scheff, S. W. (2010). Oxidative stress in the progression of Alzheimer disease in the frontal cortex. Journal of Neuropathology and Experimental Neurology, 69, 155-167.

Axelsen, P. H., Komatsu, H., & Murray, I. V. J. (2011). Oxidative stress and cell membranes in the pathogenesis of Alzheimer's disease. Physiology, 26, 54-69.

Behl, C., & Moosmann, B. (2002). Oxidative nerve cell death in Alzheimer's disease and stroke: antioxidants as neuroprotective compounds. Biological Chemistry, 383, 521-536.

Ece, A., & Pejin, B. (2015). A computational insight into acetylcholinesterase inhibitory activity of a new lichen depsidone. Journal of Enzyme Inhibition and Medicinal Chemistry, 30, 528-532.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Petersson, G. A., Nakatsuji, H., Li, X., Caricato, M., Marenich, A., Bloino, J., Janesko, B. G., Gomperts, R., Mennucci, B., Hratchian, H. P., Ortiz, J. V., Izmaylov, A. F., Sonnenberg, J. L., Williams-Young, D., Ding, F., Lipparini, F., Egidi, F., Goings, J., Peng, B., Petrone, A., Henderson, T., Ranasinghe, D., Zakrzewski, V. G., Gao, J., Rega, N., Zheng, G.,

Liang, W., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Throssell, K., Montgomery, Jr., J. A., Peralta, J. E., Ogliaro, F., Bearpark, M., Heyd, J. J., Brothers, E., Kudin, K. N., Staroverov, V. N., Keith, T., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J. C., Iyengar, S. S., Tomasi, J., Cossi, M., Millam, J. M., Klene, M., Adamo, C., Cammi, R., Ochterski, J. W., Martin, R. L., Morokuma, K., Farkas, O., Foresman, J. B., & Fox, D. J. (2016). Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford CT.

Harvey, A. L. (1995). The pharmacology of galantamine and its analogues. Pharmacology&Therapeutics, 68, 113-128.

Karadjova, V., Vakarelska-Popovska, M., & Velkov, Zh. (2021). Radical-scavenging activity characterization of a series of synthetic 3-phenylcoumarins. Computational and Theoretical Chemistry, 1202, 113300.

Marco, L., & do Carmo Carreiras, M. (2006). Galantamine, a natural product for the treatment of Alzheimer's disease. Recent Patents on CNS Drug Discovery, 1, 105-110.

Markovic, Z., Tosovic, J., Milenkovic, D., & Markovic, S. (2016). Revisiting the solvation enthalpies and free energies of the proton and electron in various solvents. Computational and Theoretical Chemistry, 1077, 11-17.

Marlatt, M. W., Lucassen, P. J., Perry, G., Smith, M.A., & Zhu, X. (2008). Alzheimer's disease: cerebrovascular dysfunction, oxidative stress and advanced clinical therapies. Journal of Alzheimers Disease, 15, 199-210.

Melo, J. B., Sousa, C., Garção, P., Oliveira, C. R., & Agostinho, P. (2009). Galantamine protects against oxidative stress induced by amyloid-beta peptide in cortical neurons. European Journal of Neuroscience, 29, 455-464.

Parameswari, A. R., & Kumaradhas, P. (2013). Exploring the conformation, charge density distribution and the electrostatic properties of galantamine molecule in the active site of AChE using DFT and AIM theory. International Journal of Quantum Chemistry, 113, 1200–1208.

Parr, R. G. (1980). Density functional theory of atoms and molecules. in: K. Fukui, B. Pullman (Eds.), Horizons of Quantum Chemistry. Academie Internationale Des Sciences Moleculaires Quantiques/International Academy of Quantum Molecular Science, vol 3. Springer: Dordrecht.

Petersen, R.B., Nunomura, A., Lee, H. G., Casadesus, G., Perry, G., Smith, M. A., & Zhu, X. (2007). Signal transduction cascades associated with oxidative stress in Alzheimer's disease. Journal of Alzheimers Disease, 11, 143-152.

Rimarcík, J., Lukes, V., Klein, E., & Ilcin, M. (2010). Study of the solvent effect on the enthalpies of homolytic and heterolytic N-H bond cleavage in p-phenylenediamine and tetracyano-p-phenylenediamine. Journal of Molecular Structure (Theochem), 952, 25–30.

Tomasi, J., Mennucci, B., & Cammi, R. (2005). Quantum mechanical continuum solvation models. Chemical Reviews, 105, 2999-3093.

Traykova, M., Traykov, T., Hadjimitova, V., Krikorian, K., & Bojadgieva, N. (2003). Antioxidant properties of galantamine hydrobromide. Zeitschrift fur Naturforschung, 58c, 361-365.

Tsvetkova, D., Obreshkova, D., Zheleva-Dimitrova, D., & Saso, L. (2013). Antioxidant activity of galantamine and some of its derivatives. Current Medicinal Chemistry, 20, 4595-4608.

Velkov, Zh., Traykov, M., Trenchev, I., Saso, L., & Tadjer, A. (2019). Topology-dependent dissociation mode of the O-H bond in monohydroxycoumarins. Journal of Physical Chemistry A, 123, 5106–5113.

Vezenkov, L. T., Georgieva, M. G., Danalev, D. L., Ivanov, T. B., & Ivanova, G.I. (2009). Synthesis and characterization of new galantamine derivatives comprising peptide moiety. Protein & Peptide Letters, 16, 1024-1028.

Vezenkov, L., Cena, H., Danalev, D., Karadjova, V., Tsekova, D., Bardarov, V., Vassilev, N. (2020). New derivatives of galantamine containing peptide fragment. Journal of Chemical Technology Metallurgy, 55, 251-260.