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Computational Radical-Scavenging Activity Investigation of Galantamine

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Abstract

Galantamine is a natural alkaloid witha proved antioxidant activity, which easily passes the blood-brain barrier. This makes it a reliable drug for the treatment of many human brain diseases.In this paper we report the results of quantum chemical calculations of the dissociationenthalpies of O-H and C-H bonds in galantamine. The results clearly show the propensity of galantamine to react with radicals in the living organisms.This includes an assessment of the stability of O-H and C-H bonds in it, as well as their acidity.In addition, hydride ion abstraction reactions such as occur with nicotine-amid-adenine-dinucleotide (NAD) are discussed here.

Introduction

Oxidative damages are the causes of many diseases, and the brain is particularly vulnerable to them because it contains easily oxidizable fatty acids, consumes large amounts of oxygen and contains low levels of endogenous antioxidants [1]. Oxidative stress plays a major role in neuronal degeneration in the brain of patients with Alzheimer's disease [2-4]. The production of reactive oxygen species exacerbates cell membrane dysfunction, [5] and this damage leads to the activation of nerve cells death [1]. Plant antioxidants with radical scavenging activity could protect people from brain diseases or at least to slow down their development, but only if they can cross the blood-brain barrier. Galantamine is a natural alkaloid that has radical scavenging properties [6] easily passes the blood-brain barrier and is relatively low toxic. This makes it a reliable drug for the treatment of many human brain diseases [7].

The antioxidant properties of galantamine hydrobromide have been proved in *in vitro* experiments [6]. The ability of galantamine and its hydrobromide to capture reactive oxygen species (ROS): $O2^{\bullet-}$, •OH and HOCl, has been attributed to the presence of the hydroxyl group at position 6 of the galantamine cyclohexene C-ring (See Figure 1). The authors come to this conclusion because any chemical transformation associated with the hydroxyl group affects the radical-scavenging activity and the latter disappears after the conversion of the OH-group to a carbonyl one. It has also been found that the radical-scavenging activity of galantamine increases when it is converted to its hydrobromide salt.

Galantamine affects many human brain pathologies [8-10]. The most significant use of galantamine is as a competitive, reversible and selective inhibitor of acetylcholinesterase, improving the cognitive functions of patients with Alzheimer's disease. It is interesting to note that it is also related to nicotine addiction [10].

The quantum chemical studies on galantamine that we found are dedicated to the distribution of its electron density and the generated electrostatic potential [11] or to the relationship between the energy of the highest occupied orbitals and their activity as acetylcholine esterase inhibitors in a group of galantamine analogues [12].

However, none of the studies looked at the ability of galantamine to enter into chemical reactions related to its most easily dissociable O-H and C-H bonds.

In this paper we will report the results of quantum chemical calculations of the enthalpies of dissociation of O-H and C-H bonds in galantamine (see Figure 1 and the Schemes 1-5 below) and norgalantamine - an analogue of galantamine without the methyl group at N11. Norgalantamine is widely used in synthetic chemistry instead of galantamine [13, 14].

The results provide an opportunity to assess their overall reactivity, their radical-scavenging activity mechanism, as well as the most probable pathways for metabolism.

Computational Protocol

The calculations were performed using the density functional theory and the hybrid Becke-3-parameter-Lee-Yang-Parr functional (DFT/B3LYP) coded in the Gaussian09 software package. [15]. The same protocol was applied earlier to another series of radical-scavengers and the results were completely adequate [16]. 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) [17].

The species (X) enthalpy is evaluated according to the 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, DH_{trans}, DH_{rot}, and DH_{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 [18, 19]. The used proton enthalpy ($\mathbf{H}_{(H^+)}$) in vacuum is 6.197 kJ mol^{-1}, and in water is – 1083.803 kJ mol^{-1}; the used enthalpy of an electron ($\mathbf{H}_{(e^-)}$) in vacuum is 3.145 kJ mol^{-1}, and in water is – 232.676 kJ mol^{-1}, the used enthalpy of a hydrogen atom ($\mathbf{H}_{(H^{\bullet})}$) in vacuum is – 1312.479 kJ mol^{-1}and in water is –1316.479 kJ mol^{-1}.

Results And Discutions

The homolytic dissociation of the X–H bond (Hydrogen Atom Transfer, HAT) is the most common mechanism in a nonpolar environment (Scheme 1). The descriptor related to the HAT mechanism is the bond dissociation enthalpy (BDE) calculated as an enthalpy difference between the obtained radical and hydrogen atom and the galantamine on the other hand: BDE = $H_{(Gal\text{-}O^{\bullet})} + H_{(H^{\bullet})} - H_{(Gal\text{-}OH)}$. The spin density in the radical obtained after homolytic dissociation of the hydroxyl group is concentrated at the oxygen atom. The Mulliken spin density at the hydroxyl oxygen is high: 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^{-1}.

The presence of the methyl group at the nitrogen at position 11 in galantamine does not affect the BDEs of the C(6)-H and O-H bonds. In norgalantamine it is almost the same – 428,44 and 321.80 kJ mol^{-1} respectively. For comparison, the BDEs of the most active hydroxyl groups in flavones and coumarins previously calculated are between 320 and 350 kJ mol^{-1} [20].

Since the HAT mechanism is expected to take place in non-polar environments, such as those in the human brain, we calculated the BDEc of the C(6)-H O-H bond in a vacuum (Table 3). It turned out that in a vacuum this bonds are a little easier dissociable than in water (Table 2).

Thus, the radical-scavenging reaction in a nonpolar ambience can be realized through homolytic dissociation of the C6-H bond. Participation of the hydroxyl group by the same HAT mechanism seems impossible for thermodynamic reasons.

Two-step mechanisms of the O-H bond dissociation are more probable in polar ambience. In the SET-PT (Single Electron Transfer–Proton Transfer) mechanism, an electron is initially detached from galantamine (norgalantamine), and a proton is detached from the resulting cation-radical in the second step (Scheme 2).

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

Electron detachment from galantamine is a significantly more probable process than the homolytic dissociation because it involves an IP of only 288.16 kJ mol^{-1}(for norgalantamine - 308.33 kJ mol^{-1}).

Proton separation from 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^{-1} (110.92 kJ mol^{-1} for the norgalantamine).

The ionization potential of the phenolic compounds we have worked with before is between 369 and up to 393 kJ mol^{-1} [20 Table 3], and the PDE is a small positive or even negative number.

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

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

The enthalpy of heterolytic dissociation of the O-H bond (PA) in galantamine is 218.26 kJ mol^{-1} (in norgalantamine - 218.18 kJ mol^{-1}), and the enthalpy of electron transfer (ETE) is 201.07 kJ mol^{-1} for both compounds. Here the first stage of SPLET - the heterolytic dissociation of the O-H bond is more difficult,

and the results obtained show that SPLET is the most probable mechanism of the O-H bond dissociation.

In order to verify the experimentally found results [6], according to which the radical-scavenging activity of the galantamine increases when it is converted to the hydrobromide salt, we calculated the PA and ETE of O-H bonds in the 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^{-1} (respectively, 210.39 kJ mol^{-1} for the norgalantamine), and the ETE for the detachment of an electron from the obtained uncharged species is 209.34 kJ mol^{-1} (respectively 209.62 kJ mol^{-1}).

PA of O-H in the galantamine with the protonated amino is about 8 kJ mol^{-1} less than that in the non-protonated, which is in agreement with the experimental results, according to which in an acidic environment galantamine is a more active radical-scavenger. On the other hand, this confirms the conclusion that the SPLET mechanism is realized in the reaction of galantamine with active radicals *in vitro*.

Dissociation of the C(6)-H bond

The C-H bond at C(6) (Scheme 1), to which the hydroxyl group is attached, should be dissociated more easily in the HAT and SET-PT mechanisms because the species obtained after the dissociation could share its spin density or charge with the neighboring carbon atoms (C7 and C8). Indeed, its radical maximum spin density is 0.676, which 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 is 321.81 kJ mol^{-1}, (and the same in norgalantamine). This is about 107 kJ mol^{-1} lower than the BDE of the O-H bond (See 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 319.78kJ mol^{-1}, and in norgalantamine: 319.65kJ mol^{-1}.

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 this second reaction becomes now 148.82 kJ mol^{-1} (148.60 kJ mol^{-1} for norgalantamine).

Let us consider the changes in the enthalpies in the two-step mechanisms of C-H bond breaking. The conversion of galantamine to a cation-radical in the SET-PT mechanism has already been considered (see Scheme 2). As said above, this is a completely feasible process from a thermodynamic point of view: IP is 288.16 kJ mol^{-1} (308.33 kJ mol^{-1} for norgalantamine). PDE of the C-H bond dissociation from the cation-radical of galanthamine is 24,457 kJ mol^{-1} (4,280 kJ mol^{-1} 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.

It should be noted that the changes in the enthalpies in both steps of the SET-PT mechanism in galantamine and in norgalantamine differ by about 20 kJ mol^{-1}.

Only the heterolytic dissociation of the C(6)-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^{-1} in galantamine (316.52 kJ mol^{-1} 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^{-1} and of the norgalantamine anion -3.61 kJ mol^{-1}).

The protonation of the amino group in galantamine and norgalantamine may benefit the SPLET mechanism (see Table 3). The enthalpy of the C-H bond heterolytic dissociation (PA) in the protonated galantamine is 295.83 kJ mol^{-1} (in the protonated norgalantamine: - 295.87 kJ mol^{-1}). ETE of the anions with a protonated amino group are bigger negative numbers: - 14.67 kJ mol^{-1}(and - 14.63 kJ mol^{-1}).

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:

The charge in these positions can be shared effectively.

The change in the enthalpy of this process at C(6) is 319,72kJ mol^{-1} (in norgalantamine – 318,48kJ mol^{-1}). The same dissociation at C(12) adjacent to the nitrogen atom, is more probable: 228,51kJ mol^{-1} for galantamine and 237,69 kJ mol^{-1} 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 – 202,62kJ mol^{-1}, and from morphine, where the change in enthalpy is only 187,72 kJ mol^{-1}.

Conclusion

Enthalpies of the dissociation of O-H and C-H bonds in galantamine and its demethylated analogue – norgalantamine, by different mechanisms were calculated. These reaction enthalpies are suitable descriptors for radical-scavenging activity as well as for the propensity to participate in other reactions that may occur with these compounds in the living organisms. It was found that the most probable mechanism of dissociation of the O-H bond in them is SPLET, and the protonated galantamine and norgalantamine have a lower enthalpy of dissociation than the non-protonated. These results correspond to the experimental radical-scavenging activity of galantamine and confirm the adequacy of our results. The results obtained may be useful to explain the diverse biological activity of galantamine, as well as to indicate the mechanism of its metabolism.

References

1. Behl C, Moosmann B (2002) Oxidative nerve cell death in Alzheimer's disease and stroke: antioxidants as neuroprotective compounds. Biol Chem 383(3-4):521-536. DOI: 10.1515/BC.2002.053
2. Ansari MA, Scheff SW (2010) Oxidative stress in the progression of Alzheimer disease in the frontal cortex. J Neuropathol Exp Neurol 69(2):155-167. DOI: 10.1097/NEN.0b013e3181cb5af4
3. Marlatt MW, Lucassen PJ, Perry G, Smith MA, Zhu X (2008) Alzheimer's disease: cerebrovascular dysfunction, oxidative stress and advanced clinical therapies. J Alzheimers Dis 15(2):199-210.doi: 10.3233/jad-2008-15206
4. Petersen RB, Nunomura A, Lee HG, Casadesus G, Perry G, Smith MA, Zhu X. (2007) Signal transduction cascades associated with oxidative stress in Alzheimer's disease. J Alzheimers Dis 11(2):143-152.DOI: 10.3233/jad-2007-11202
5. Axelsen PH, Komatsu H, Murray IVJ (2011) Oxidative stress and cell membranes in the pathogenesis of Alzheimer's disease. Physiology 26(1):54-69. DOI: 10.1152/physiol.00024.2010
6. Traykova M, Traykov T, Hadjimitova V, Krikorian K, Bojadgieva N (2003)Antioxidant Properties of Galantamine Hydrobromide. Z Naturforsch 58c: 361-365.DOI: 10.1515/znc-2003-5-613
7. 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. DOI: 10.2174/09298673113209990148
8. Marco L, do Carmo Carreiras M, (2006) Galanthamine, a natural product for the treatment of Alzheimer's disease. Recent Pat CNS Drug Discov 1(1):105-110.DOI: 10.2174/157488906775245246
9. Melo JB, Sousa C, Garção P, Oliveira CR, Agostinho P (2009) Galantamine protects against oxidative stress induced by amyloid-beta peptide in cortical neurons. Eur J Neurosci 29(3):455-464. DOI: 10.1111/j.1460-9568.2009.06612.x
10. Harvey AL (1995) The pharmacology of galanthamine and its analogues. Pharmacol Ther 68(1):113-128. DOI: 10.1016/0163-7258(95)02002-0
11. Azhagesan Renuga Parameswari and Poomani Kumaradhas (2012) Exploring the Conformation, Charge Density Distribution and the Electrostatic Properties of Galanthamine Molecule in the Active Site of AChE Using DFT and AIM Theory. International Journal of Quantum Chemistry DOI: 10.1002/qua.24251
12. Abdulilah Ece and Boris Pejin (2015) A computational insight into acetylcholinesterase inhibitory activity of a new lichen depsidone. J Enzyme Inhib Med Chem 30(4):528-32. doi: 10.3109/14756366.2014.949256.
13. Vezenkov LT, Georgieva MG, Danaleva DL, Ivanova TB, Ivanova GI (2009) Synthesis and Characterization of New Galanthamine Derivatives Comprising Peptide Moiety. Protein & Peptide Letters16(9):1024-8. doi: 10.2174/092986609789055412.
14. Vezenkov L, Cena H, Danalev D, Karadjova V, Tsekova D, Bardarov V, Vassilev N (2020) New Derivatives of Galantamine Containing Peptide Fragment. J Chem Technol Metallurgy 55 (2):251-260

15. Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016. http://gaussian.com/
16. Velkov Zh, Traykov M, Trenchev I, Saso L, Tadjer A (2019) Topology-dependent dissociation mode of the O-H bond in monohydroxycoumarins. J Phys Chem A 123 (24):5106–5113. https://doi.org/10.1021/acs.jpca.9b00535.
17. Tomasi J, Mennucci B, Cammi R (2005) Quantum Mechanical Continuum Solvation Models. Chem Rev 105 (8):2999-3093.https://doi.org/10.1021/cr9904009
18. Rimarˇcík J, Lukeˇs V, Klein E, Ilˇcin 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. J Mol Struct Theochem 952 (1-3):25–30.DOI: 10.1016/j.theochem.2010.04.002
19. Markovic Z, Tošovic J, Milenkovic D, Markovic S (2016) Revisiting the solvation enthalpies and free energies of the proton and electron in various solvents. Comput Theor Chem 1077:11-17. doi.org/10.1016/j.comptc.2015.09.007.
20. 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. https://doi.org/10.1016/j.comptc.2021.113300

Tables

Table 1 Enthalpies of O-H and C-H bond dissociations (in kJ mol^{-1}, in water)

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

Table 2 BDE in a vacuum

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

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

	PA_{O-H}	$ETE_{O-anion}$	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

Schemes

Schemes 1, 2, 3, 4 and 5 are available in the Supplementary Files section.

Figures

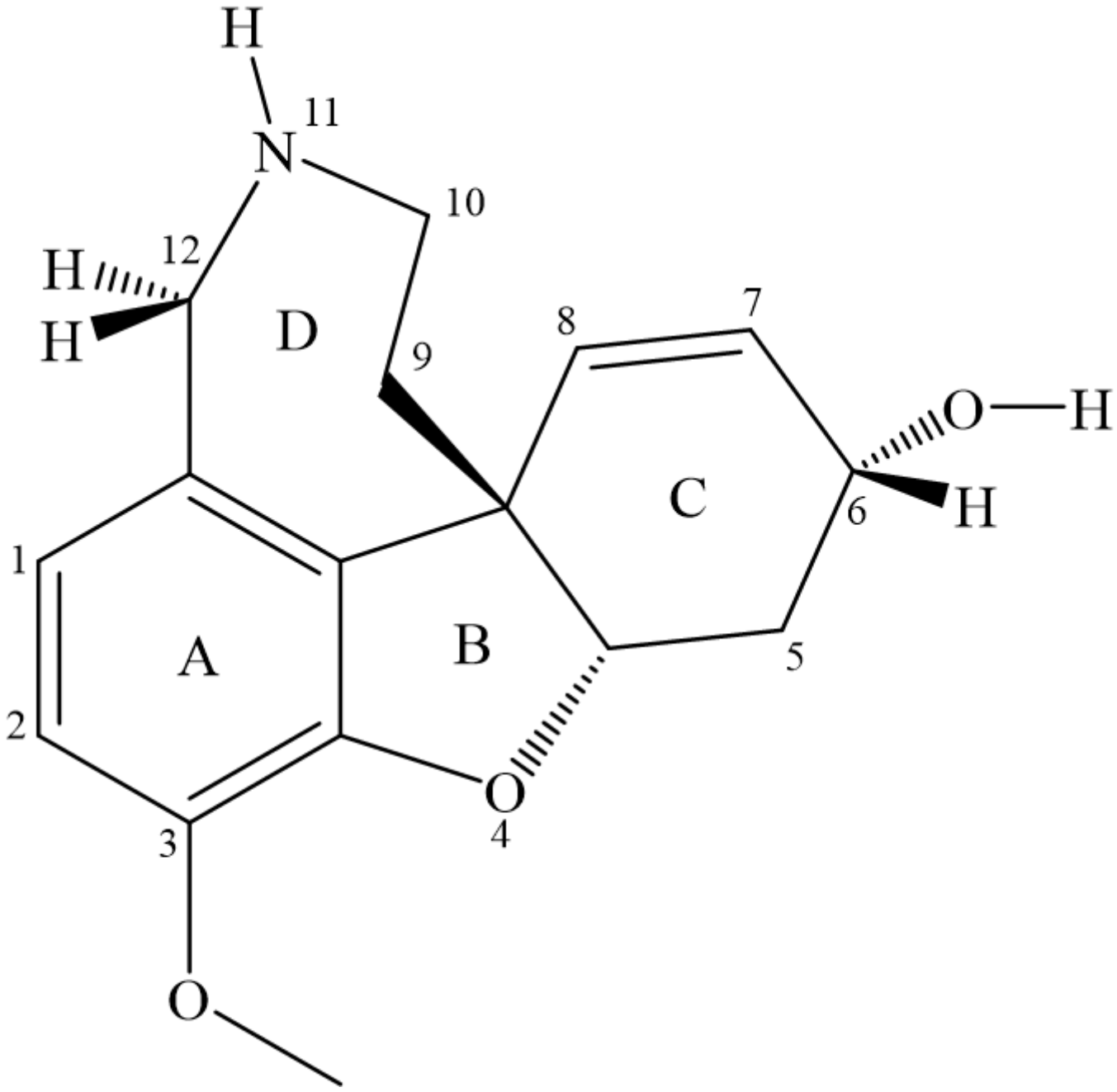

Figure 1

Galanthamine

Supplementary Files

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- Scheme3..docx

- Scheme4..docx
- Scheme5..docx