

# Modeling the properties of new potential modifiers of the medium for cryopreservation of fish sperm

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**Abstract.** To model the antioxidant activity (antiradical activity against the hydroxyl radical and superoxide anion radical) of a number of new heteroatomic organic compounds containing OH and NH groups, calculations were performed within the framework of density functional theory using the B3LYP functional. The energy effects of reactions of the studied compounds with hydroxyl radical and superoxide anion radical were calculated. Based on the calculations performed for a series of 12 compounds, leading compounds were identified for which high antiradical activity with respect to both radicals is predicted. These leading compounds can be considered as promising for use as an exogenous antioxidant additive to the basic cryoprotective medium to increase the cryoresistance of fish sperm.

## 1 Introduction

Today, cryopreservation of fish spermatozoa is one of the promising methods both in the strategy of preserving genetic biodiversity and in aquaculture, the main source of formation and maintenance of stocks of endangered and economically valuable fish species [1]. Currently, the significant potential of using cryotechnology has not been realized due to the low fertility of defrosted sperm as a result of their damage during cryopreservation procedures.

Numerous studies indicate the important role of reactive oxygen species (ROS) in cryodamage to fish reproductive cells [2], which are normal metabolic products and in small concentrations play an important role in the physiology of these cells [3]. However, at high concentrations they have a toxic effect, causing oxidative damage to important biomolecules, including proteins, which leads to the inactivation of enzymes associated with sperm motility [4]. The concentration of ROS in fish sperm is determined by the balance between the rates of their formation and neutralization by the antioxidant defense system.

It is known that during cryopreservation at the freezing and defrosting stages, the

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concentration of ROS in fish sperm increases. Thus, it has been reported that in fish sperm there is a hyperproduction of the initial ROS – superoxide anion radical  $O_2^{\cdot -}$  (SOAR) [5], from which other, more aggressive oxygen metabolites can be formed [6], including the most aggressive form of ROS – hydroxyl radical  $HO^{\cdot}$  (HR). These processes lead to an imbalance in the oxidant-antioxidant system and the development of oxidative stress, since the endogenous antioxidant protection of mature spermatozoa is limited by the low content of cytoplasm.

Fish sperm are particularly susceptible to oxidative damage because the phospholipids of biomembranes are dominated by polyunsaturated fatty acids, which are substrates for lipid peroxidation [7]. It is also necessary to take into account the decrease in the concentration of antioxidant components of fish sperm during cryopreservation due to the dilution of sperm with a cryoprotective medium. Moreover, the ability of sperm to repair molecular damage is limited because such cells are terminally differentiated and transcriptionally inactive. Therefore, although spermatozoa are more resistant to cold shock than other cell types [8], they are damaged during cryopreservation, which leads to a decrease in their fertility.

Taking into account the above factors, one of the strategies for improving the quality of gametes during cryopreservation is the introduction of antioxidants of various natures into the basic cryogenic environment [9]. However, to date, the use of exogenous antioxidant supplements to improve the quality of defrosted fish sperm is still controversial, since there are known facts indicating a negative effect of compounds with antioxidant activity on the quality of defrosted sperm [10]. Currently, the possibility of using hybrid antioxidants with multiple mechanisms of antioxidant action is being studied to increase the cryoresistance of fish sperm [11-13].

The greatest protective activity was demonstrated by multifunctional phenolic antioxidants, which are capable of protecting reproductive cells at various stages and phases of oxidative stress. In this regard, in order to identify compounds with antioxidant activity capable of increasing the cryoresistance of fish sperm, a preliminary assessment of the antioxidant activity of potential antioxidants with cryoprotective action is necessary. For such a preliminary assessment, it is possible to use quantum-chemical model calculations, which allow one to study at the electronic level the mechanism of interaction of various antioxidants with free radicals, the hyperproduction of which is observed during cryopreservation, and to identify leading compounds, which will make it possible to substantiate the feasibility of further research into their antioxidant and protective activity during cryopreservation of spermatozoa of commercially valuable fish.

The aim of this work is quantum-chemical modeling by the density functional method of the antioxidant activity of a number of new heteroatomic organic compounds containing OH and NH groups. The specific task of the work is to model the mechanism of interaction of these compounds with the superoxide anion radical and hydroxyl radical and to quantitatively assess the antiradical activity of the studied antioxidants in relation to these radicals. In the model calculations, known synthetic antioxidants were used as reference compounds: 2,6-di-*tert*-butyl-4-methylphenol (ionol, BHT) and a water-soluble analogue of vitamin E, trolox.

## 2 Research methods

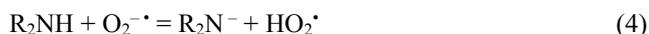
The new hybrid heteroatomic compounds studied in this work (Table 1) were synthesized using previously known methods [14, 15]. To assess the potential antioxidant properties of the studied compounds, quantum chemical modeling was used, implemented using the Gaussian 09 computer program. The calculations were performed using the density functional method. The following functional and basis were used: B3LYP/6-31++G(d,p). Quantum chemical calculations were carried out with full optimization of the geometry of all structures under consideration.

The potential antioxidant activity of the studied compounds was assessed using their reactions with HR and SOAR as an example. For the model reactions under consideration, energy effects ( $\Delta E$ ) were calculated. The value of  $\Delta E$  was calculated as the difference between the total energies of the final and initial structures. The  $\Delta E$  value was considered as an indicator and criterion of the ease of the simulated reactions and the stability of the radicals and anions formed from the initial structures of antioxidants. The more negative the  $\Delta E$  value, the easier the modeled reaction proceeds and the higher the potential level of the corresponding type of antioxidant activity.

### 3 Results and discussion

Modeling of antioxidant activity towards superoxide anion radical and hydroxyl radical was carried out for a series of 12 new, recently synthesized, hybrid heteroatomic compounds, the names of these compounds are given in Table 1. Well-known phenolic antioxidants such as ionol and trolox were considered as control compounds.

The antioxidant and, in particular, antiradical activity of the compounds under consideration was assessed by modeling their reactions with the superoxide anion radical and hydroxyl radical, which proceed according to schemes (1) – (4):



When antioxidants interact with SOAR, an alternative route is theoretically possible, in which not a proton, but a hydrogen atom is detached from the center of antioxidant activity (OH or NH group), which is described by scheme (5).



However, as calculations have shown, such an alternative route is much less probable from the point of view of the energy of the process and requires energy expenditure, in contrast to the main route (detachment of a hydrogen ion), in which energy is usually released. For example, in the case of compound **3**, for the reaction proceeding according to scheme (3), the obtained  $\Delta E$  value was -118.2 kJ/mol, while for the reaction according to scheme (5), the calculated  $\Delta E$  value was +70.4 kJ/mol.

**Table 1.** Names and some structural features of the studied compounds.

Compound number	Name	Structural features	Anti-radical centers
1	6-[[3,5-Di( <i>tert</i> -butyl)-2-hydroxyphenyl](hydroxy)methyl]-3,4-dihydro-2,5-benzodiazocin-1(2 <i>H</i> )-one	Phenol-containing derivative of 2,5-benzodiazocin-1(2 <i>H</i> )-one	OH groups, NH group
2	6-[[3-(1-Adamantyl)-2-hydroxy-5-methylphenyl](hydroxy)methyl]-3,4-dihydro-2,5-benzodiazocin-1(2 <i>H</i> )-one	Phenol-containing derivative of 2,5-benzodiazocin-1(2 <i>H</i> )-one	OH groups, NH group
3	3,5-Di-( <i>tert</i> -butyl)-4-hydroxybenzenecarbaldehyde N-(2-oxo-1,2-dihydro-3 <i>H</i> -indol-3-ylidene)hydrazone	Phenol-containing indole derivative	OH group, NH group
4	4-Methyl-N'-(2-oxo-1,2-dihydro-3 <i>H</i> -indol-3-ylidene)benzenesulfonohydrazide	Derivative of 4-methylbenzenesulfonic acid with an indole fragment	NH groups
5	6,3-Di( <i>tert</i> -butyl)-4 <i>b</i> ,9 <i>b</i> -dihydroxy-4 <i>b</i> ,9 <i>b</i> -dihydro-10 <i>H</i> -indeno[1,2- <i>b</i> ][1]benzofuran-10-one	Dihydroxy derivative of indeno[1,2- <i>b</i> ]benzofuran-10-one	OH groups
6	6-(1-Adamantyl)-4 <i>b</i> ,9 <i>b</i> -dihydroxy-8-methyl-4 <i>b</i> ,9 <i>b</i> -dihydro-10 <i>H</i> -indeno[1,2- <i>b</i> ]benzofuran-10-one	Dihydroxy derivative of indeno[1,2- <i>b</i> ]benzofuran-10-one	OH groups
7	3-[( <i>E</i> )-3-(2,4-Dihydroxyphenyl)-2-propenoyl]-2 <i>H</i> -chromen-2-one	Phenol-containing derivative of coumarin	OH groups
8	3-[( <i>E</i> )-3-(4-Hydroxyphenyl)-2-propenoyl]-2 <i>H</i> -chromen-2-one	Phenol-containing derivative of coumarin	OH group
9	Methyl N-(4-[( <i>E</i> )-3-[3,5-di( <i>tert</i> -butyl)-4-hydroxyphenyl]-2-propenoyl]phenyl)carbamate	Phenol derivative with phenylcarbamate moiety	OH group, NH group
10	Methyl N-(4-{4-[4-(4-methoxyphenyl)-1-methyltetrahydro-1 <i>H</i> -pyrrol-3-yl]carbonyl}phenyl)carbamate	Pyrrole derivative with a phenylcarbamate moiety	NH group
11	5-Acetyl-4-hydroxy-1,3-thiazine-2,6-dione semicarbazone	Hydroxyl-containing derivative of thiazine	OH group, NH groups, NH <sub>2</sub> group
12	Methyl N-[4-(3,5-dioxo-2,5-dihydro-3 <i>H</i> -imidazo[5,1- <i>α</i> ] isoindol-1-yl)-3-hydroxyphenyl]carbamate	Isoindole derivative with a phenylcarbamate moiety	OH group, NH groups

The obtained results of model calculations for compounds **1–12** and control substances (ionol and trolox) are presented in Table 2.

Compounds **1** and **2** are phenol-containing derivatives of an eight-membered heterocyclic compound, 2,5-benzodiazocin-1(2*H*)-one, and differ in the substituents in the phenol fragment. Both compounds contain three centers of potential antiradical activity: a phenolic OH group, an alcoholic OH group, and an NH group of a heterocycle.

**Table 2.** Calculated values of  $\Delta E$ , kJ/mol.

Compound	Anti-radical center	$\Delta E$ for reactions with HR according to schemes (1), (2)	$\Delta E$ for reactions with SOAR according to schemes (3), (4)
1	Ph-OH	-158.1	-34.4
1	>CH-OH	-42.3	-51.6
1	>NH	-34.5	-15.4
2	Ph-OH	-133.3	-29.7
2	>CH-OH	-47.5	-31.9
2	>NH	-32.0	+5.0
3	Ph-OH	-171.3	-118.2
3	>NH	-91.1	-26.0
4	Ph-NH-CO-	-89.9	-68.3
4	=N-NH-SO <sub>2</sub> -	-115.3	-90.9
5	>C(OH)-Ph	-68,0	-34,1
5	>C(OH)-O-Ph	-149,1	-42,5
6	>C(OH)-Ph	-67.8	-65.4
6	>C(OH)-O-Ph	-158.6	-42.4
7	Ph-OH, OH group at position 2 of the benzene ring	-138.8	-96.3
7	Ph-OH, OH group at position 4 of the benzene ring	-132.8	-104.7
8	Ph-OH	-142.8	-102.3
9	Ph-OH	-175.4	-116.9
9	>NH	-87.4	-39.6
10	>NH	-86.1	-34.2
11	-OH	-132.9	-193.8
11	-NH-CO-	-68.6	-112.1
11	=N-NH-	-144.5	-134.5
11	-NH <sub>2</sub>	-18.7	-112.9
12	Ph-OH	-168.4	-143.6
12	Ph-NH-	-94.4	-76.1
12	>N-CO-NH-	-167.9	-135.5
Ionol	Ph-OH	-173.2	-29.0
Trolox	Ph-OH	-179.7	-1.5
Trolox	-COOH	-146.3	-57.6

As calculations show, the phenolic hydroxyl group (Ph-OH) is a more preferable center for binding the hydroxyl radical compared to the alcoholic OH group (>CH-OH) and NH group. The  $\Delta E$  values for the phenolic OH group in the case of compounds **1** and **2** are -158.1 and -133.3 kJ/mol, respectively, while for the other two centers the  $\Delta E$  values are significantly higher – by about 100 kJ/mol (Table 2). This result can be explained by the high stability of phenoxyl radicals formed from compounds **1** and **2** during the reaction with HR according to scheme (1).

In relation to SOAR, compounds **1** and **2** exhibit moderate activity –  $\Delta E$  values are in the range from -51.6 to +5.0 kJ/mol.

Compound **3** contains two potential antiradical centers in its structure: the OH group of a fragment of a sterically hindered phenol (Ph-OH) and the NH group of a heterocycle. The obtained results show that compound **3** can be considered as one of the leaders in the studied series of substances. For the Ph-OH center of this compound, fairly low (below -100 kJ/mol)

$\Delta E$  values were obtained for both the reaction with HR and the reaction with SOAR (-171.3 and -118.2 kJ/mol, respectively). The structure of compound **3** creates favorable conditions for high stability of the radical and anion formed during reactions at the Ph–OH center according to schemes (1) and (3), respectively. The high stability of the phenoxyl radical and phenolate anion formed from compound **3** is facilitated by the presence of three substituents in the benzene ring, in particular, two *tert*-butyl groups are in the *ortho* positions. The unsaturated substituent located in the *para* position of the ring contains a large system of conjugated bonds, which creates additional opportunities for delocalization of the unpaired electron and negative charge.

Compound **4** does not contain OH groups, and two NH groups can be considered as potential centers of antiradical activity, for one of which a fairly low  $\Delta E$  value (-115.3 kJ/mol) was obtained for the reaction with the hydroxyl radical.

Compounds **5** and **6** are structural analogues with two hydroxyl groups, they are derivatives of benzofuran and differ in substituents in one of the benzene rings. The obtained calculation results demonstrated significant differences in the predicted antioxidant activity of the two OH groups in the case of a reaction with a hydroxyl radical. Thus, for compound **6**, the  $\Delta E$  values for the reactions of two different OH groups with HR were -67.8 and -158.6 kJ/mol. The calculations performed provide an explanation for this difference in the properties of the two OH groups: in the case of an OH group with a lower  $\Delta E$  value, the radical formed according to scheme (1) undergoes isomerization with the formation of a stable, sterically hindered phenoxyl radical.

Compounds **7** and **8** represent another pair of structural analogues, these are coumarin derivatives that differ in the number of OH groups in the benzene ring. Compound **7** contains two OH groups in the *ortho* and *para* positions of the ring, while the structure of compound **8** contains one OH group in the *para* position. As expected, the results obtained for all three Ph–OH centers in compounds **7** and **8** were close. For all three OH groups, a fairly high activity is observed in reactions with both HR and SOAR; the obtained  $\Delta E$  values are at the level of -100 kJ/mol and below.

The structure of compound **9** has common features with the structure of compound **3** – it is a sterically hindered phenol with a bulky unsaturated substituent in the *para* position, creating additional opportunities for delocalization of the unpaired electron and negative charge. For the Ph–OH center of compound **9**, low  $\Delta E$  values were obtained, allowing one to predict high activity in reactions with both HR and SOAR (-175.4 and -116.9 kJ/mol, respectively). Thus, compound **9**, along with compound **3**, can also be classified as a leading compound in terms of predicted antioxidant and antiradical activity, within the framework of the model approach used.

Compound **10** does not contain OH groups, its structure has one potential active center – the NH group in the phenylcarbamate fragment. For this NH group,  $\Delta E$  values above -100 kJ/mol were obtained, which allows us to predict a relatively moderate level of activity in reactions with HR and SOAR, in comparison with other substances of the studied series.

Interesting results were obtained for compound **11**, which contains four potential centers of antiradical activity (OH and NH groups) in its structure. In this compound,  $\Delta E$  values below -100 kJ/mol – both for the reaction with HR and for the reaction with SOAR – were obtained for two centers at once – the OH group of the heterocycle and one of the NH groups. The structure of compound **11** contains a large conjugated system and nine heteroatoms, which creates favorable conditions for the formation of stable radicals and anions in accordance with schemes (1) – (4).

Compound **12** is an isoindole derivative with a hydroxyphenylcarbamate moiety. The results of the modeling allow us to predict a sufficiently high level of activity in relation to the hydroxyl radical and superoxide anion radical for two centers – the phenolic OH group and one of the NH groups.

Summarizing the obtained data, compounds **3**, **9**, **11** and **12** should be singled out as leading compounds in terms of the probable level of antioxidant and antiradical activity. For these compounds, calculations predict high activity in reactions with both HR and SOAR.

It should be noted that for compounds **3**, **9** and **12**, calculations predict antiradical activity towards the hydroxyl radical at the level of such well-known antioxidants as ionol and trolox. Thus, for phenolic OH groups of ionol and trolox, the  $\Delta E$  values for reactions with HR are, respectively, -173.2 and -179.7 kJ/mol, while for compounds **3**, **9** and **12**, similar  $\Delta E$  values range from -168.4 to -175.4 kJ/mol (Table 2).

Moreover, as follows from the data in Table 2, the majority of antioxidants studied in this work are not inferior to ionol and trolox (or surpass these control substances) in predicted activity in relation to SOAR.

## 4 Conclusion

Thus, in the present work, the antioxidant activity of a series of new hybrid heteroatomic compounds was modeled with respect to two active forms of oxygen – hydroxyl radical and superoxide anion radical. The modeling was carried out using the quantum-chemical density functional method. Antioxidant and, in particular, antiradical activity is due to the presence in the structure of the studied compounds of OH and NH groups, which are capable of reacting with active forms of oxygen. It has been shown that for many compounds from the series under consideration, a high level of antioxidant activity comparable to known effective antioxidants can be predicted.

The obtained results can be used in the search for new antioxidants intended for modifying the cryogenic environment in order to increase the cryostability of fish reproductive cells during low-temperature preservation.

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