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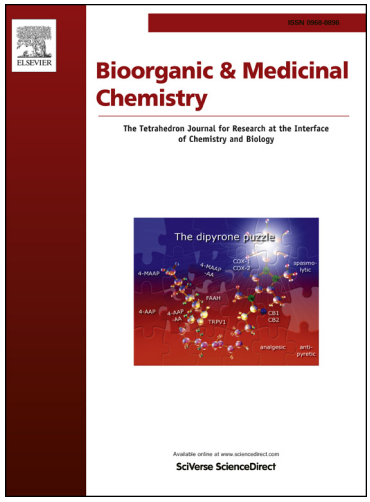

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Synthesis, electronic properties, antioxidant and antibacterial activity of some new benzimidazoles

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Abstract

Two groups of benzimidazole derivatives were synthesized using as precursors 5(6)-substituted 2-mercapto-benzimidazol-thiols and their antioxidant activity was investigated using TBA-MDA test.

In the group of 1,3-disubstituted-benzimidazol-2-imines the highest lipid peroxidation inhibition effect 74.04 % (IC_{50} = 141.89 μg/mL) revealed ethyl [3-(2-ethoxy-2-oxoethyl)-2-imino-5-benzoyl-2,3-dihydro-1H-benzimdazol-1-yl]acetate **12** while in the group of 2-substituted-1,3-

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thiazolo[3,2-a]benzimidazolones the highest inhibition effect showed 2-(4-fluorobenzylidene)-7-(phenylcarbonyl)[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one **17** 90.76% (IC_{50} = 53.70 μg/mL). In order to estimate the capability of the studied benzimidazoles to act as radical scavengers the structure of the most active derivative within the both subseries was optimized at B3LYP/6-311++G** level and the respective bond dissociation enthalpies were calculated. The appropriate models for the HAT and SET-mechanism of the antioxidant activity were proposed.

The antibacterial activity of the compounds was evaluated against two Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017). 1,3-Diphenylpropyl-5-methyl-1,3-dihydro-2H-benzimidazol-2-imine **14** exhibited significant activity against *B. subtilis*, *S. aureus*, *S. abony* and ***E. coli*** (with MIC values of 0.125, 0.016, 0.50 and 0.50 mg/mL, respectively). The group of thiazolobenzimidazolones did not reveal antibacterial activity against the tested strains.

Keywords: 2-imino-benzimidazoles; 1,3-thiazolo[3,2-a]benzimidazolones, lipid peroxidation inhibition, antibacterial activity, DFT calculations

1. Introduction

Benzimidazole heterocycle system is an important pharmacophore and privileged structure in the medicinal chemistry. Its derivatives and particularly 2-aminobenzimidazoles exert various biological activities such as antioxidant,[1] antimicrobial,[2] anthelmintic,[3] anticancer,[4] anti-hypertensives,[5] antiviral,[6] and antifungal.[7] The benzimidazole ring system exists in the structure of many antiparasitic, anthelmintic, antifungal, antiviral and antitumor drugs. Some of them as albendazole, omeprazole, lansoprazole, bendamustine etc. have found widespread application in medicinal practice. Therefore the synthesis of new benzimidazoles and the study of their biological properties are of pharmacological interest.

Many benzimidazoles derivatives were synthesized and proven for their antibacterial activity.[8,9] Benzimidazoles, containing thiadiazole or oxadiazole rings in 1-position were studied for antibacterial and antifungal activity. All of the reported benzimidazoles showed good activity towards Gram-positive bacteria and negligible activity towards Gram-negative bacteria.[10,11] A series of 2-substituted-5-nitro-benzimidazole derivatives showed comparable activity to that of ampicilline against Gram-positive bacteria *S. aureus*, *S. mutans*, *B. subtilis* and to the activity of nalidixic acid against Gram negative *E. coli*, *S. typhi* and *P. aeruginosa*.[12] Furthermore the 2-aminobenzimidazole dimers are identified as a novel scaffold that possesses antibacterial activity and as such may aid in the discovery of new antibacterial agents.[13] Benzimidazolopeptides as well as head-to-head bis-benzimidazoles have shown good antimicrobial activity against pathogenic fungal strains and Gram negative bacterial strains.[14, 15]

A series of benzimidazoles, containing 1,3,4-thiadiazole or 1,2,4-triazole rings were synthesized and tested for antioxidant properties by using various *in vitro* systems.[16] Albendazole, substituted with benzoyl group in 1-position, possesses significant antioxidant activity.[17] Thiazolo[3,2-a]benzimidazole derivatives inhibited the oxidation of adrenaline to adrenochrome, and prevented the formation of the superoxide radical.[18] The antioxidant potential of some coumarine-benzimidazole hybrids as radical scavengers was evaluated.[19] 2-Aryl-1-arylmethyl-1*H*-benzimidazoles can be considered as potential antioxidant and xanthine oxidase inhibitory agents.[20]

It is known that active oxygen species as superoxide, hydroxyl and peroxyl radicals are causes of the oxidative stress associated with the pathogenesis of various diseases as Alzheimer's, Parkinson's, cataracts and DNA damage leading to carcinogenesis. Taking into account that the antioxidants and enzymes take part of the protective system of the organism it is of interest to synthesize new benzimidazole derivatives and to study their antioxidant properties.

On the other site the spreading out of various resistant bacterial and fungal strains versus the antibacterial drugs is a challenge to researchers to create and develop structurally new class of

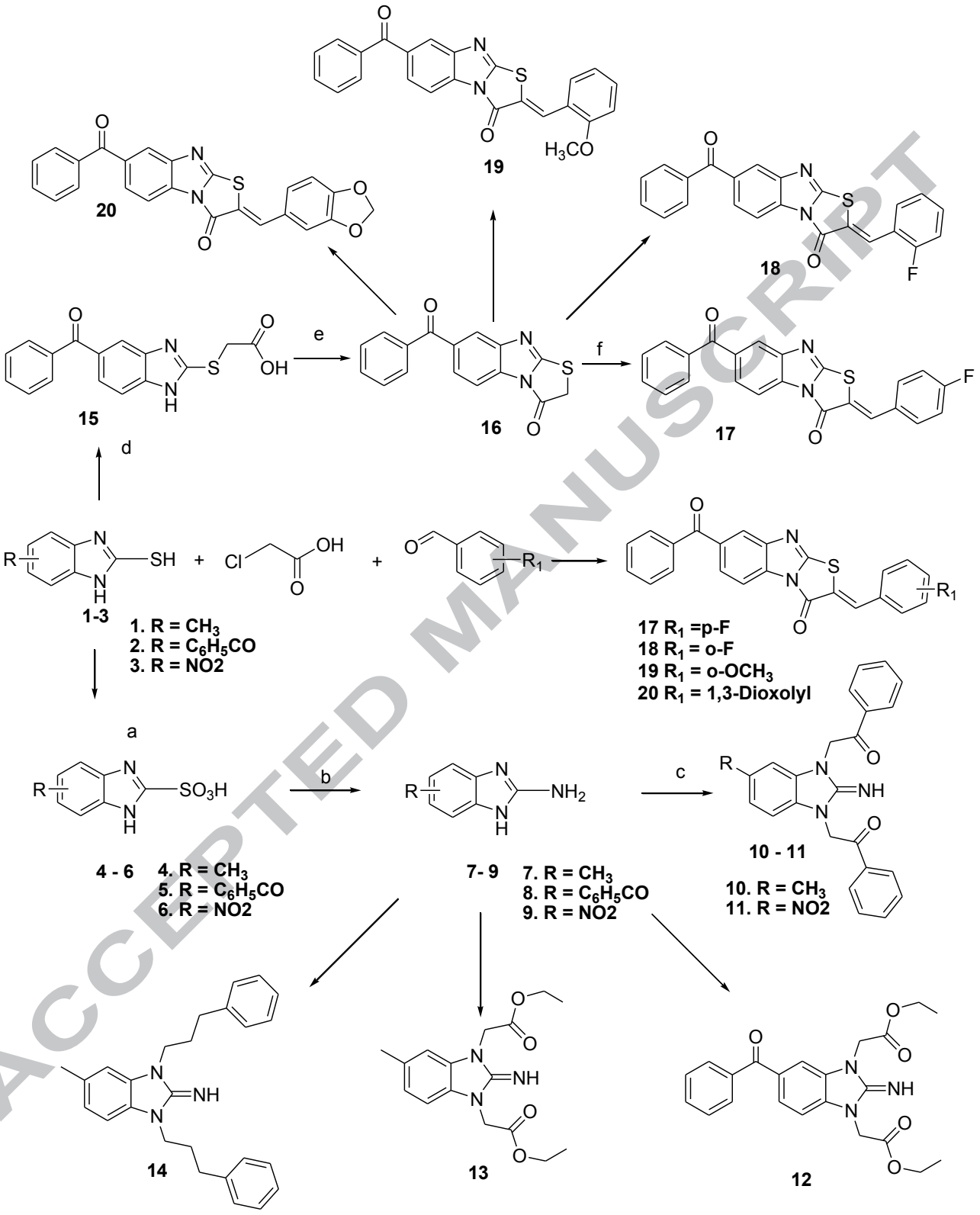
molecules with new mechanisms of action. Thus the generation of new antibacterial agents as well as antioxidants is of special interest in the field of medical chemistry. Therefore, the aim of the present study was to synthesize two groups of benzimidazole derivatives using substituted benzimidazol-2-thiols as starting compounds, 2-imino-2,3-dihydro-benzimidazole and 1,3-thiazolo[3,2-a]benzimidazolone derivatives, to examine their antibacterial and antioxidant properties and to perform SAR analysis using DFT calculations.

2. Results and Discussions

2.1. Chemistry

Two groups of some new benzimidazole derivatives were synthesized by use of 2-mercapto-benzimidazoles as precursors, and their antibacterial and antioxidant properties were investigated. The nucleophilic substitution of 2-amino-benzimidazoles **7-9** with halogen derivatives was performed under solid-liquid phase transfer catalysis conditions in dry acetonitrile and resulted in bis-1,3-disubstituted-2,3-dihydro-benzimidazole **10-14**, obtained in good yields (65-86 %). The second group of compounds was obtained by the reaction of thiazolobenzimidazolone **16** and substituted arylaldehydes using pyridine as catalyst in good yields. The one-pot synthesis requires prolonged reaction time, but the yields of the obtained end product are higher.

The synthesis of 1,3-thiazolo[3,2-a]benzimidazolones and 2,3-dihydro-2-iminobenzimidazoles is illustrated and outlined in Scheme 1.



Scheme 1. Synthesis of the studied benzimidazole compounds. Reagents and conditions: a) $KMnO_4$, NaOH water solution, refluxing; b) NH_4OH, 150 °C in welded ampoule; c) TBAB, dry K_2CO_3, halogen derivative, 25 °C, acetonitrile; d) $ClCH_2COOH$, NaOH/ethanol, refluxing 3 h; e)

$(CH_3CO)_2O$, pyridine medium refluxing 10 min; aromatic aldehydes, cat. Py, ethanol, refluxing 4-6 h.

The starting 4-substituted benzimidazolyl-2-thiols were synthesized by refluxing of 4-substituted-1,2-diamino-benzenes, carbon disulfide, ethanol and sodium hydroxide according to the method described in our earlier report.[21] The Williamson reaction of the thiols with chloroacetic acids in ethanol in the presence of excess of sodium hydroxide led to the corresponding thioacetic acids. The cyclocondensation of thioacetic acids with acetic anhydride in pyridine medium at 100 ^oC resulted to 1,3-thiazolo[3,2-a]benzimidazolones. The compounds **17-20** were synthesized by the use of two alternative methods. The first one, condensation between thiazolo[3,2-a]benzimidazolones and aromatic aldehydes performed in ethanol gave the 1,3-thiazolo[3,2-a]benzimidazolones substituted at the 2-position. By the second method compound **17-20** were synthesized by Knovenagel condensation and subsequent cyclocondensation of the starting thiol, chloroacetic acid and the appropriate aromatic aldehydes.

The 5-substituted-2-amino-benzimidazoles **7-9** were synthesized by oxidation of 5-substituted-2-mercaptobenzimidazoles with water solution of $KMnO_4$ in the presence of sodium hydroxide to yield benzimidazol-2-yl-sulphonic acids, followed by the treatment of the sulphonic acids with ammonium hydroxide in welded ampoule for 5 hours at 145-150 °C.[22] The introduction of substituents in 1-th and 3-th position of the 5-substituted 2-aminobenzimidazoles was performed under solid-liquid phase transfer catalysis conditions in dry acetonitrile using dry potassium carbonate and different halogen derivatives. Except for compound **12**, the synthesis of all other benzimidazole derivatives is reported here for the first time.

The chemical structures of the compounds were established by elemental analyses, IR and 1H NMR spectra and the results are presented in the Experimental part. The elemental analyses indicated by the symbols of the elements were within ± 0.4% of theoretical values.

2.2. Pharmacology

2.2.1. Antibacterial activity

Antibacterial activity was evaluated against two Gram-positive and three Gram-negative bacteria. Gram-positive bacteria used were: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538 while Gram-negative bacteria utilized in the assay were: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017. The antibacterial properties of the studied compounds was determined by using a broth microdilution method in 96 multi-well microtitre plates.

Compounds **10-14** and **17-20** were examined as potential antimicrobial agents, starting from initial 2 mg/mL concentration for all tested samples. The minimal inhibitory concentrations (MICs) ranged from 0.016 to 1.0 mg/mL. Only three among the tested samples, i.e. compounds **10**, **11** and **14** showed antimicrobial activity (Table 1). Compound **14** was the most active one being effective against four bacteria, especially against *S. aureus* with MIC value of 0.016 mg/mL. According to available data[23] compounds **14** and **10** might be considered as antimicrobial agents with strong activity while compound **11** belongs to the group of agents with moderate activity. The tested samples were less effective than the antibiotic used as a reference standard. Comparing the antibacterial activities of the group of 1,3-disubstituted-2-imino-benzimidazoles can be concluded that the compounds possessing ester groups (**12**, **13**) at positions 1 and 3 do not exhibit antibacterial activity in contrast to those containing phenylethanone or phenylpropyl groups as substituents. It could be supposed that the presence of phenylpropyl groups at positions 1 and 3 of 2-imino-benzimidazol core is responsible for the potent antibacterial activity of the compound **14.** This compound possesses much higher lipophilicity than the other studied 1,3-disubstituted-2-imino-benzimidazoles as well as the smallest topological surface area (see Table 3). Comparing the activities of compounds **10** and **11** can be observed that the presence of methyl group at position 5 of

2-imino-benzimidazol core is more favorable for antimicrobial activity than the presence of nitro group. The 2-substituted-1,3-thiazolo[3,2-a]benzimidazolones **17-20** did not show antibacterial activity, therefore their results were not included in Table 1.

Table 1. Minimal inhibitory concentrations (MIC values given in mg/mL, unless stated otherwise) of compounds **10-14**

Samples	*B. subtilis*	*S. aureus*	*S. abony*	*E. coli*	*P. aeruginosa*
10	0.50	0.50	n.a.	n.a.	n.a.
11	1.00	n.a.	n.a.	n.a.	n.a.
12	n.a.	n.a.	n.a.	n.a.	n.a.
13	n.a.	n.a.	n.a.	n.a.	n.a.
14	0.125	0.016	0.50	0.50	n.a.
Doxycycline (μg/mL)	1.56	0.78	6.25	0.78	12.50
DMSO (10%)	n.a.	n.a.	n.a.	n.a.	n.a.

2.2.2. Antioxidant activity

Lipid peroxidation (LP), as well as its inhibition in the presence of the synthesized compounds, was measured by using TBA–MDA test. [24,25]

Lipid peroxidation (LP), as well as its inhibition in the presence of the synthesized studied benzimidazole derivatives, was measured by using TBA–MDA test, the method based on the MDA (LP secondary product) reaction with TBA to obtain a red coloured complex with maximum absorption at 530 nm The TBA-MDA complex absorbances in the supernatant read at 530 nm were used to calculate the inhibition percentage of lipid peroxidation[26] by using the equation given in the experimental section, and the results are given in Table 2. All samples were assayed for LP

inhibitory activity at concentrations of 200 μg/mL in the fiinal reaction mixture. Those showing inhibition greater than 50% at this concentration were tested in a broader concentration range to allow calculation of IC_{50} values. The IC_{50} values represent the concentrations of studied benzimidazoles in the reaction mixture which induce a 50% LP-inhibition effect after 3h incubation.

Table 2. Lipid peroxidation inhibition effects of studied benzimidazoles **10-14** and **17-20** (example concentration 200 μg/mL) and IC_{50} values (given in μg/mL)

Comp No	**Lipid Peroxidation Inhibition Effect**		**Standard antioxidants**	**Lipid Peroxidation Inhibition Effect**	
	(%)	IC_{50} (μg/mL)		(%)	IC_{50} (μg/mL)
10	39.26±3.11	> 200	*Trolox*	85.61 ± 2.05	7.74
11	23.43±2.79	> 200	*Quercetin*	91.12 ± 0.36	10.47
12	74.04±0.90	141.89	*Caffeic acid*	99.98 ± 2.52	3.79
13	55.87±3.16	176.58	*L-Ascorbic acid*	99.90 ± 1.70	9.12
14	62.15±5.71	144.88			
17	90.76±1.04	53.70			
18	75.76±6.78	86.97			
19	80.35±2.08	74.11			
20	60.80±5.35	85.51			

The obtained results indicated that most of the tested compounds of both groups possessed good antioxidant properties. The thiazolo[3,2-a]benzimidazolones demonstrated higher activity than the 1,3-disubstituted-benzimidazol-2-imines. Highest inhibition percentage of lipid peroxidation was expressed by 2-(4-fluorobenzylidene)-7-(phenylcarbonyl)[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one **17** (IC_{50} = 53.70 μg/mL). The next by activity is compound **19** containing a methoxygroup at o-position of the benzene ring with IC_{50} value of 74.11 %. The presence of the F-atom in 2-nd position

of benzene nucleus as in compound **18** led to decrease of lipid peroxidation inhibition (IC_{50} = 86.97 μg/mL).

In the group of 1,3-disubstituted-2-imino-benzimidazoles compound **12** showed the most effective LP inhibition (IC_{50} = 141.89 μg/mL)**.**

2.3. SAR

Three different mechanisms could be regarded when concerning how antioxidants deactivate the free radicals – H-atom abstraction (HAT mechanism, see Eq. 1),[27-30] electron transfer (SET mechanism, see Eq. 2a-2b),[30-33] and sequential proton loss electron transfer (SPLET mechanism, see Eq. 3a-3b)[27,34]:

$R\text{-}H \rightarrow R^{\bullet} + H^{\bullet}$ (1)

$R\text{-}H \rightarrow R^{+\bullet} + e^{-}$ (2a)

$R^{+\bullet} \rightarrow R^{\bullet} + H^{+}$ (2b)

$R\text{-}H \rightarrow R^{-} + H^{+}$ (3a)

$R^{-} \rightarrow R^{\bullet} + e^{-}$ (3b)

The efficacy of the antioxidant to react via HAT is characterized by the bond dissociation enthalpy (BDE) – the lower BDE values, the higher the radical scavenging capacity of R-H. In the SET mechanism, the reactivity is evaluated by ionization potential (IP). A lower IP implies an easier electron release. The first step of the SPLET mechanism consists in formation of an anion and thus the proton affinity (PA) of the formed anion is used as a measure for the reactivity. Lower PA values indicate an easier extraction of the proton. The net result of the three radical scavenging mechanisms is the same. SET and SPLET mechanisms are favoured in polar environment because the generated charged species are stabilized by the solvent. Based on calculation of the reaction enthalpies for each

of the mechanisms, it is possible to suggest the most probable mechanism of action of a compounds particular group.[27]

In order to estimate the capability of the studied iminobenzimidazoles **10-14** to act as radical scavengers via HAT mechanism the structure of the most active derivative within the series (compound **12**) was optimized at B3LYP/6-311++G** level and the respective bond dissociation enthalpies were calculated. The most favourable positions for hydrogen atom abstractions are expected to be the two α-C atoms (*site 1* and *site 2*, Fig. 1) from the side chains and the imino group N-H (*site 3*, Fig. 1). It is known that the BDE of the α-C-H bonds in alkylamines decrease with increasing number of alkyl sibstituents on the N and C atoms.[35] In compounds **10-13** the α-C-H bonds are additionally polarized by the adjacent ester groups.

According to the B3LYP/6-311++G** calculations, the most favorable position for hydrogen atom abstraction is *site 1* (Figure 1).

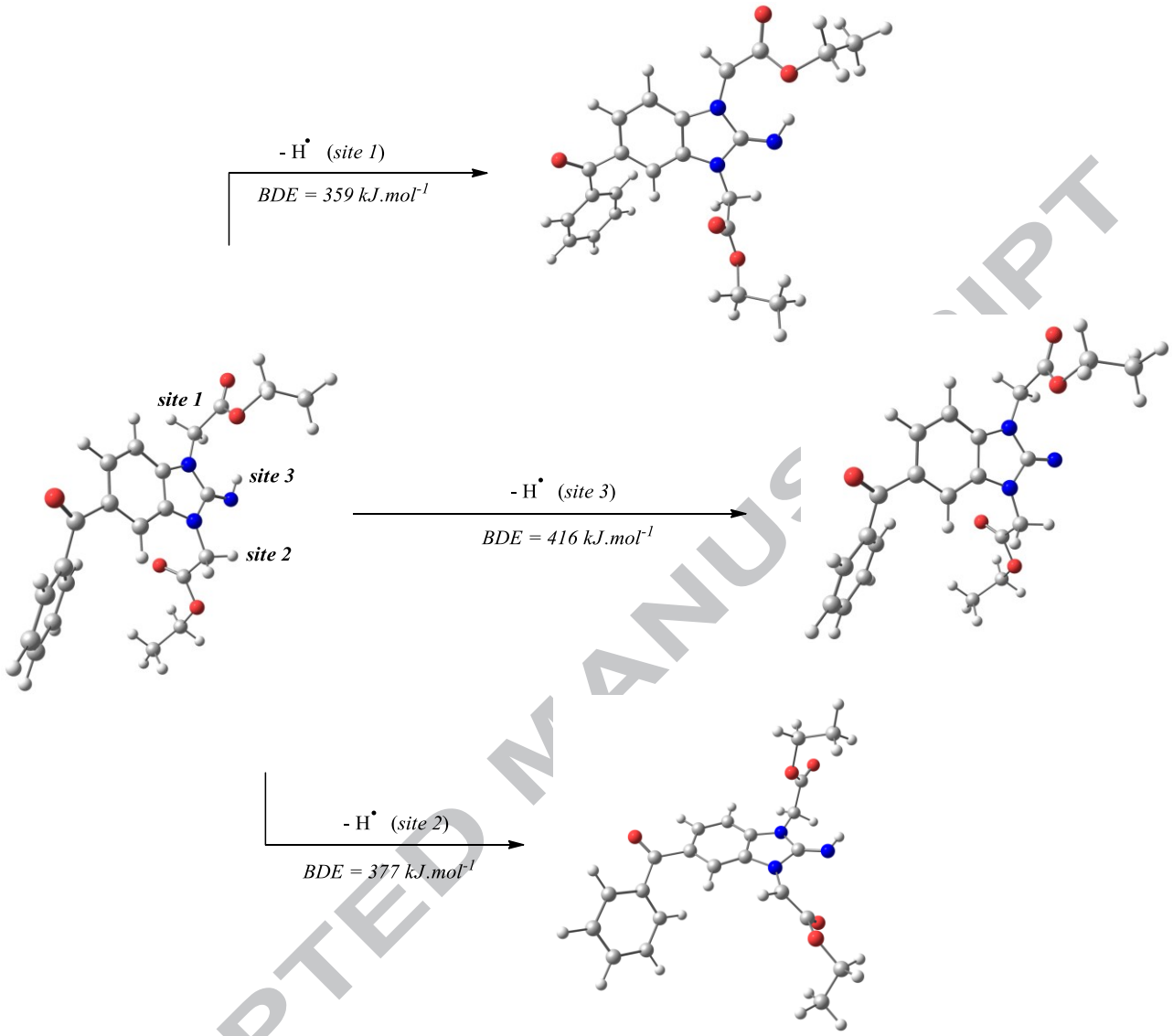


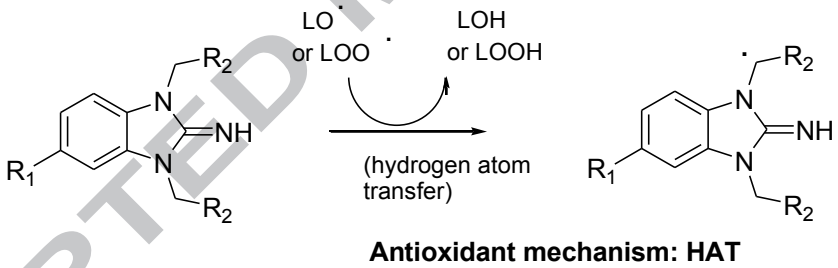
Figure 1. Hydrogen atom abstraction at different sites and corresponding DFT B3LYP/6-311++G** bond dissociation enthalpies of compound **12** in kJ/mol.

In order to prevent effectively lipid peroxidation the antioxidants should have a lower BDE value than the free lipid radicals. LOO· radicals typically display a BDE of about 367 kJ/mol, as reported based on radical kinetics, gas-phase acidity cycles, and photoionization mass spectrometry measurements.[36] However, a lipid BDE value, representative for the chosen level of theory is needed to enable an accurate comparison. For this purpose the BDE of O-H bond of methanol was calculated in gas-phase:

$BDE(CH_3OH) = H(CH_3O^{\cdot}) + H(H^{\cdot}) - H(CH_3OH) = 413\ kJ/mol$

Effective chain-breaking antioxidants such as α-tocopherol show substantially lower BDE - 327 kJ/mol according to the calculations in gas-phase the same computational scheme.[27]

Comparing the BDE value calculated for methanol with those of compound **12**, the N-H bond should be excluded as probable site for HAT reaction. The BDE values related to the formation of C-centered radicals are lower than those of the N-centered one by 40 kJ/mol. The atomic spin population analysis show that the formation of C-centered radicals is associated with more efficient delocalization of the odd electrons through the conjugated system than in the case of the N-centered one. This leads to better radical stability for the C-centered radicals, and especially those formed by HAT from *site 1*. Based on the obtained enthalpies the following hypothetical mechanism could be suggested:



Scheme 2. Hypothetical mechanism of antioxidant action of compounds **10-14**.

The compounds in the second studied subseries of benzimidazoles **17-20** possess an aromatic structure without any OH and NH groups. Compounds **17-20** might exist in two diastereoisomeric forms (Z and E) concerning the relative orientation of the phenyl rings and the thiazolone fragment. According to the B3LYP/6-311++G** optimization of the structure of the most active derivative within the series **17**, the difference between the ZPVE-corrected total energies of the more stable Z-isomer and the less stable E-isomer is 16.9 kJ/mol and therefore E-isomer is not expected to be present in solution or solid state. The NMR data show no evidence of that isomer. The only possible

site for H-abstraction is the benzylidene group (Fig. 2), thus feasibility of the HAT mechanism was estimated based on the respective BDE. As it can be seen in Fig. 2, the calculated value is 421 kJ/mol which is higher than those of $CH_3O^{\bullet}$, excluding the HAT as possible mechanism of action.

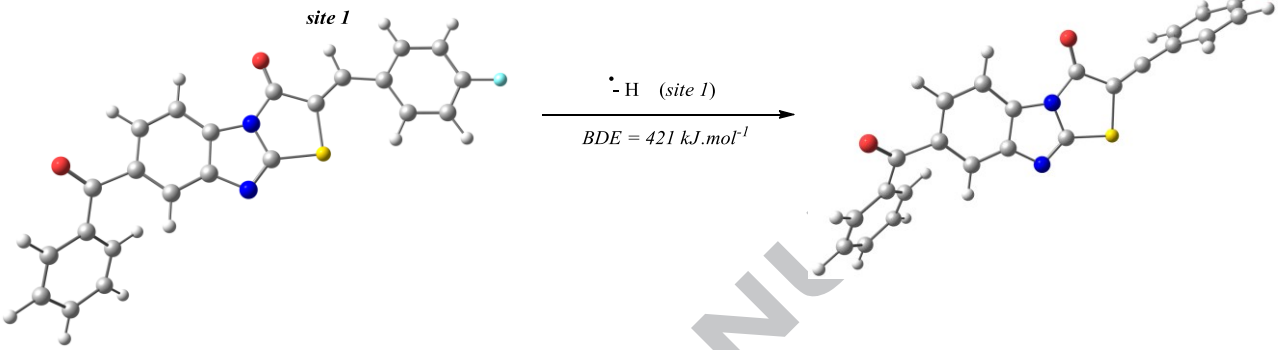
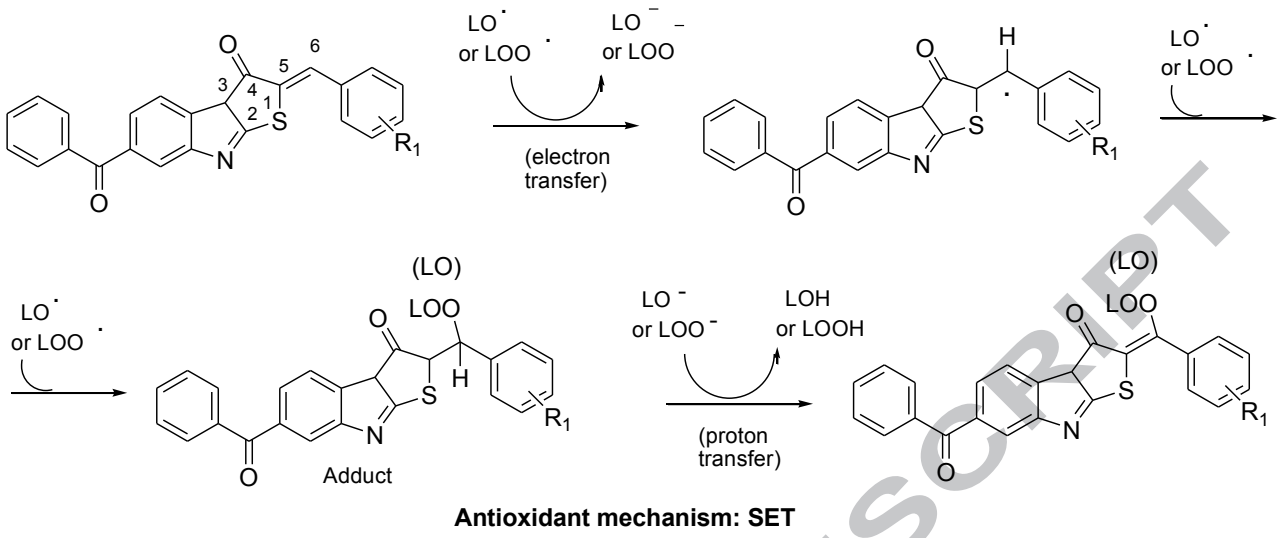


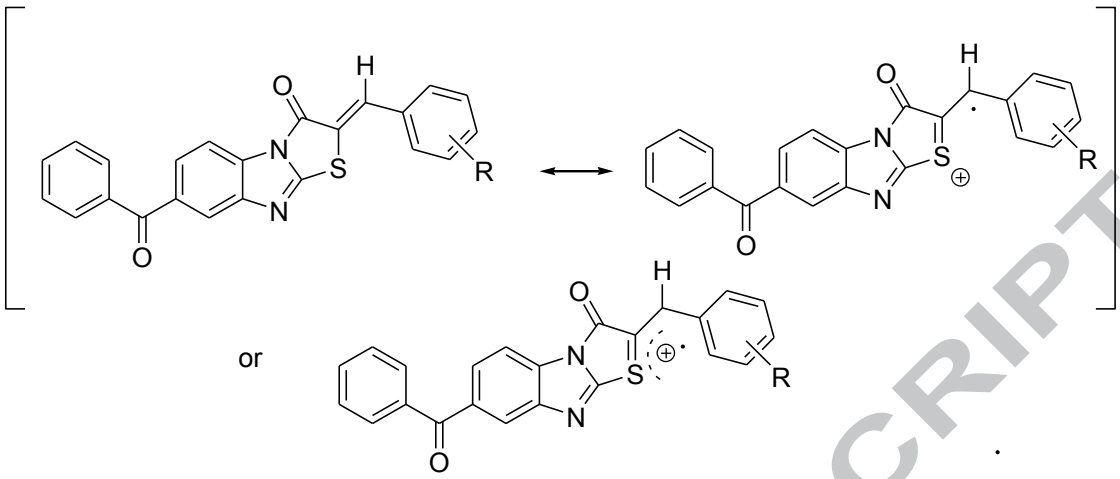
Figure 2. Hydrogen atom abstraction and corresponding DFT B3LYP/6-311++G** bond dissociation enthalpy of **17** in kJ/mol.

On the other hand, compounds **17-20** might easily donate an electron due to the presence of their extended conjugated system. They resemble in structure to some recently investigated 2-amino-5-alkylidenethiazol-4-ones[26] and pyrrolopyrimidines[37] exhibiting antioxidant action. Similarly to these cases, a mechanism of stepwise oxidation could be suggested for compounds **17-20** (Scheme 3). These compounds could produce radical cations able to scavenge the lipid alkoxyl ($LO^{\bullet}$), lipid peroxyl ($LOO^{\bullet}$) or hydroxyl ($^{\bullet}OH$) radicals by initial electron transfer (SET mechanism), then form an intermediate adduct and terminate the process by a proton transfer (Scheme 3):



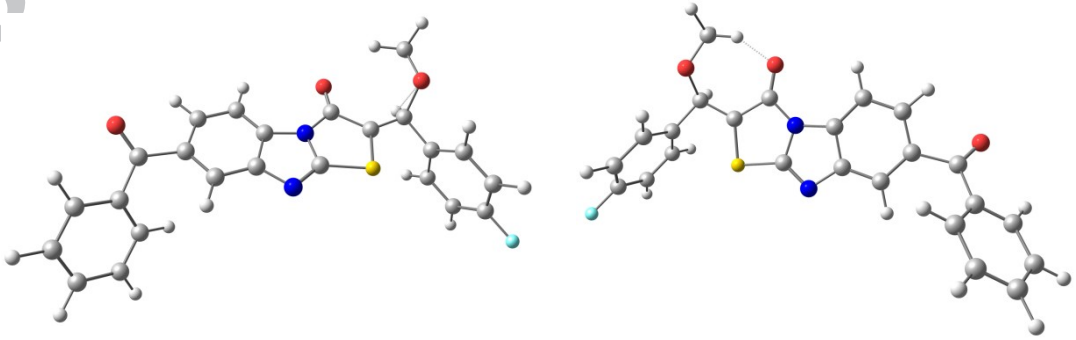
Scheme 3. Hypothetical mechanism of antioxidant action of compounds **17-20**.

The structure of the radical cations and adducts formed in the course of the antioxidant action according to Scheme 3, were further investigated by modeling compound **17** and including a methoxy radical to represent the lipid. The optimized geometry of the radical cation shows a planar orientation of the phenyl ring and thiazolobenzimidazolone fragment. The S1-C5 bond is substantially shortened and the C5-C6 bond is much longer compared to the parent neutral molecule. The analysis of the spin densities over the atoms indicates that approximately one third of unpaired electron is localized in the fragment S1-C5-C6 and could be illustrated by the resonance structures showed on Scheme 4.



Scheme 4. Resonance structures of the peroxyl scavengers (radical cations) of compounds **17-20**.

Thus the attack of the lipid radicals would be expected at S1, C5 and C6. The adducts formed by addition of methoxy radical at these sites were studied by optimizing the geometry of the relevant cation products of compound **17**. According to the calculated ZPVE-corrected total energies, addition at C6 seems to be the most favorable. The adduct has tetrahedral structure due to the sp^3 hybridization of C6 with two possible stereo configurations – S and R (Fig. 3). In the final step of the proposed antioxidant reaction, after transferring a proton to the lipid anion, the molecule would restore its planarity.



A B

Figure 3. Stereo isomers of the methoxy adduct of compound **17** – S configuration at C6 (A) and R configuration at C6 (B).

In view of future medical application, the promising pharmacological potential of studied benzimidazoles demonstrated above, should match also favorable pharmacokinetical behavior in living organisms i.e. sufficient bioavailability and transportation through different membranes to the site of action, optimal process of metabolization and elimination. In this relation the prediction of molecular properties such as lipophilicity, molecular size, flexibility and presence of hydrogen-donor and acceptors could provide useful preliminary information.

Table 3. Calculated molecular properties of compounds **10-14** and **17-20** for assessment of the drug-likeness

Compd. No.	**$m_i$logP[a]**	**TPSA[b]**	**N_{atoms}[c]**	**MW[d]**	**N_{ON}[e]**	**N_{OHNH}[f]**	**$N_{viol.}$[g]**	**$N_{rotb.}$[h]**	**Vol[i]**
Rule	**< 5**			**< 500**	**< 10**	**< 5**		**(< 10)**	
10	2.83	67	29	383	5	1	0	6	352
11	2.34	113	31	414	8	1	0	7	358
12	2.02	103	30	409	8	1	0	10	367
13	1.01	86	23	319	7	1	0	8	293
14	5.18	33	29	383	3	1	1	8	381
17	5.30	51	29	400	4	0	1	3	329
18	5.25	51	29	400	4	0	1	3	329
19	5.15	60	30	412	5	0	1	4	350
20	5.03	69	31	426	6	0	1	3	348

[a] octanol-water partition coefficient, calculated by the methodology developed by Molinspiration; [b] polar surface area; [c] number of nonhydrogen atoms; [d] molecular weight; [e] number of hydrogen-bond acceptors (O and N atoms); [f] number of hydrogen-bond donors (OH and NH groups); [g] Number of "Rule of five" violations; [h] number of rotatable bonds; [i] molecular volume.

Based on the analysis of a large number of drugs, Lipinski and coworkers established an effective methodology for estimation of potential drug solubility and permeability.[38] The required molecular properties were conveniently set into a "Rule of five": poor absorption or permeation are more likely to occur when the molecule has molecular weight more than 500, log P over 5, and contains more than 5 H-bond donors or 10 H-bond acceptors. More than one violation of the rule is the critical limit for acceptable drug-likeness.

The physico-chemical properties of the studied benzimidazoles, calculated using Molinspiration tool,[39] are shown in Table 3. Data indicate that none of the compounds is above the critical limit established by the "Rule of five". Molinspiration methodology for calculation of miLogP implements fragment-based contributions and correlation factors which makes it robust and widely applicable tool. Compounds **10-13** show miLogP values below 5 which is favorable feature for their oral bioavalability. The highest lipophilicity among the group of 1,3-disubstituted-2-imino-benzimidazoles is observed with compound **14** that contains only phenylpropyl substituents without any polar groups. The derivatives containing thiazolobenzimdazolone moiety **17-20** show significantly greater lipophilicity with miLogP values slightly above 5. Their higher lipophilicity is due to their smaller polar surface area which is another useful descriptor of the oral bioavailability[40] and drug transport properties.[41,42] It is being expressed here as topological surface area (TPSA) which is a sum of the surface areas occupied by oxygen and nitrogen atoms and the hydrogens attached to them. TPSA represents the hydrogen bonding capacity of the molecules. Molecules with TPSA less than 140 $Å^2$ are recognized to have good intestinal absorption, and those with TPSA less than 60 $Å^2$ show good blood-brain barrier penetration.[41,42] All presented benzimidazoles are

expected to have good intestinal absorption and most of them – also good blood-brain barrier penetration.

On the other hand, all compounds containing N-alkyl substituents show higher conformational flexibility manifested through their greater number of rotatable bonds. The number of rotatable bonds is an important factor for the efficient binding to receptors and channels as well as for the oral bioavailability.[40] Molecules with more than 10 rotatable bonds tend to show poor oral bioavailability. All studied compounds, satisfy this criterion and compounds **17-20** have low conformational flexibility with less than 5 rotatable bonds. The benzimidazole moiety itself has no rotatable bonds and could be regarded as a rigid linker.

Hydrogen bonding capacity of the drug candidates is also described by the number of H-bond donors and acceptors. The compounds in the series show 3 to 8 H-bond acceptors, but none (**17-20**) or one (**10-14**) H-bond donor. Molecular volumes of the compounds in the series are less than 400 $Å^3$. Summarizing the physico-chemical properties of studied benzimidazoles, we could conclude that they obey the "Rule of five" and meet all criteria for good solubility and permeability.

3. Conclusion

Two groups of benzimidazole derivatives were synthesized by use of 2-mercaptobenzimidazoles as precursors in order to estimate their antioxidant and antibacterial properties. The results obtained by the screening showed that derivatives of 2-iminobenzimidazoles **10** and **14** possessed strong activity against *B. subtilis*, *S. aureus*, *S. abony* and *E. coli* whereat their MIC_{50} were in the range 0.016 - 0.50 mg/mL. The thiazolo[3,2-a]benzimidazolones demonstrated higher activity than the 1,3-disubstituted-benzimidazol-2-imines The 2-substituted thiazolobenzimidazoles **17** and **19** revealed very strong lipid peroxidation inhibition effects with IC_{50} values of 53.70 μg/mL and 74.11 μg/mL, respectively. SAR analysis was performed and some electronic properties of the compounds were established using B3LYP/6-311++G** calculations. On the basis of calculated bond dissociation enthalpies, it was suggested that iminobenzimidazoles **10-**

14 might act as radical scavengers via hydrogen atom abstraction preferably in the α-C atom of the side chains attached to the iminobenzimidazole rings. Based on the higher BDE for the alkilydene group in benzimidazoles **17-20**, the HAT was excluded as possible mechanism of action of these compounds. It was suggested that due to their electron-donating properties, compounds **17-20** might undergo a stepwise oxidation via SET mechanism and produce radical cations able to scavenge the lipid alkoxyl ($LO^{\bullet}$), lipid peroxyl ($LOO^{\bullet}$) or hydroxyl ($^{\bullet}OH$) radicals.

4. Experimental

4.1. Chemistry

Melting points (mp) were determined on an Electrothermal AZ 9000 3MK4 apparatus and were uncorrected. IR spectra were recorded on a Bruker spectrophotometer with potassium bromide discs and as well as on aVarian 660-IR, FT-IR Spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II+ 250 MHz and a Bruker Avance II+ 600 MHz NMR instrument. The spectra are referred to the solvent signal. Chemical shifts are expressed in ppm and coupling constants in Hz. The precise assignment of the ^{1}H and ^{13}C NMR spectra was accomplished by measurement of 2D homonuclear correlation (COSY), DEPT-135 and 2D inverse detected heteronuclear (C–H) correlations (HMQC and HMBC). The microanalyses for C, H, N and S were performed on Perkin-Elmer elemental analyzer. The reactions were monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F254, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm).

The starting 4-substituted-1,2-diaminobenzenes were commercial available.

The thiols **1-3** as well as the 2-sulphonic acids and the 2-aminobenzimidazoles were synthesized according to the procedure described by us earlier[21,22]:

*4.1.1. 5-Methyl-1H-benzimidazole-2-thiol **1***: Yield –71.3 %; Mp >300°C; IR, KBr, (cm^{-1}): νNH 3426; νAr 3126; νSH 2570; δAr 1634, 1618, 1562; δCH_3 1372; νC-S 1118; δAr 799; ^{1}H- NMR

(DMSO-d6) δ (ppm): 13.12 (s, 1H,SH), 10.48 (s, 1H, NH), 7.89 (s, 1H, 9-H, Ar), 7.53 (m, 2H, 6-H, 7-H, J = 8.45), 2.66 (s, 3H, CH_3); Analysis: Calc. for $C_8H_8N_2S$: C, 58.51; H, 4.91; N, 17.06; S, 19.52; Found: 58.48; H, 4.95; N, 17.16; S, 19.60.

6.1.2. (2-Mercapto-1H-benzimidazol-5-yl)(phenyl)methanone **2**: Yield – 96,8 %; Mp – 248- 250 °C; Rf = 0.68, mobile phase: ^{1}H NMR (DMSO-d6) δ (ppm): 1.877 (s, 1H, SH), 7.251 (d, J=8.2 Hz, 1H, 7-H), 7.462 (d, J=1.6 Hz, 1H, 4-H), 7.545 (dd, J=8.2 Hz, J=1.6 Hz, 1H, 6-H), 7.558 (t, J=7.7 Hz, 2H, m-Ph), 7.658 (dt, J = 7.4 Hz, J=1.4 Hz, 1H, p-Ph), 7.700 (dd, J=8.1 Hz, J=1.4 Hz, 2H, o-Ph), 12.75 (bs, 1H, NH); ^{13}C NMR (DMSO-d6) δ (ppm): 109.30(7-C), 110.81(4-C), 125.12(6-C), 128.48(m-Ph), 129.35(o-Ph), 130.51(3a-C), 132.12(p-Ph), 132.92(7a-C), 137.14(5-C),137.99(i-Ph), 170.78(C=N), 195.08(C=O); Analysis: Calc. for $C_{14}H_{10}N_2OS$; C, 66.12; H, 3.96; N, 11.02; O, 6.29; S, 12.61; Found: C, 66.09; H, 3.91; N, 11.12; O, 6.32; S, 12.64.

4.1.3. 5-Nitro-1H-benzimidazole-2-thiol **3**: Yield – 84%; Mp – 245-247°C; Rf - 0.41, mobile phase: $CHCl_3$/ethyl acetate = 6:1. ^{1}H NMR (DMSO-d6) δ (ppm): 7.94(dd, 1H, CH, J = 9.10 Hz); 8.13 (m, 1H, CH); 8.34 (d 1H), 12.32(s, 1H, SH); Analysis: Calculated for $C_7H_5N_3O_2S$: C, 43.07; H, 2.58; N, 21.53; O, 16.39; S, 16.43; Found: C, 43.17; H, 2.53; N, 21.57; O, 16.41; S, 16.42.

4. 2. General procedure for compounds 10-14

To a solution of 5(6)-substituted-2-aminobenzimidazole (0.01 mol) in dry acetonitrile (50 mL) was added anhydrous potassium carbonate (2.7 g, 0.02 mol) and tetrabutylammonium bromide (TBAB) (0.9 g, 0.003 mol) and 0.02 mol of the halogen organic reagent was dropped by cooling. The mixture was stirred for 8-10 hours vigorously at 25 ºC and monitored by TLC over the reaction period. After completing the reaction, the mixture was filtered to separate the solid K_2CO_3, and the organic solvent was removed under vacuum.The residue was then crystallized from appropriate solvent to give the relevant compounds **10-14**.

4.2.1. 2-[2-Imino-5-methyl-3-(2-oxo-2-phenylethyl)-2,3-dihydro-1H-benzimdazol-1-yl]-phenylethanone **10**: Yield 69%; Mp. 283-285 °C; Rf - 0.71, mobile phase $CHCl_3$/CH_3OH - 8:2; IR,

KBr, (cm^{-1}): νNH 3335; νC=O 1689; δNH 1662; ^{1}H NMR (DMSO-d6) δ (ppm): 2.367 (s, 3H, CH_3); 5.992 (s, 4H, $2CH_2$); 7.133 (d, 2H, Ar); 7.630 (t, 3H, Ar), 7.813 (m, 3H, Ar); 8.076 (m, 2H, Ar); 8.131 (m, 3H, Ar); 8.834 (m, 2H, NH_2^+); ^{13}C NMR (DMSO-d6) δ (ppm): 20.98 (C-7); 52.04(C-10, C-14); 128.18(C-9); 128.18(C-9); 128.51(C-6); 12895(C-8); 133.64 (C-19, C-23, C-25); 127.83 (C-20, C-22, C-26, C-28); 133.64 (C-21, C-27); 134.10 (C-18, C-24); 151.08(C=N); 191.04 (C-11, C-15); Analysis: Calc. for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96; O, 8.35; Found: C, 75.20; H, 5.49; N, 10.98; O, 8.39.

4.2.2. 2-[2-Imino-5-nitro-3-(2-oxo-2-phenylethyl)-2,3-dihydro-1H-benzimdazol-1-yl]-1-phenylethanone **11:** Yield 82%, Mp. 246°C, Rf - 0.71, mobile phase CH_2Cl_2/CH_3OH - 8:1; IR, KBr, (cm^{-1}): νNH 3338; νC=O 1679; δNH 1652; δNO_2 1335; δAr 751, 691; ^{1}H NMR (DMSO): 5.732(s, 4H, $2CH_2$); 7.445(s, 1H,Ar); 7.636(m, 4H, Ar); 7.752(t, 2H, Ar); 8.072(s, 4H, Ar); 8.119(s, 1H, Ar); ^{13}C NMR (DMSO-d6) δ (ppm): 48.04(C-10, C-14); 104.59(C-9); 108.43(C-6); 118.31(C-7); 128.4(C-20, C-24); 129.02(C-26, C-30); 130.00 (C-29, C-27); 134.26(C-22, C-28); 134.35(C-4); 134.38(C-5); 135.34(C-19, C-20), 142.18(C-NO_2); 154.35(C=N); 191.67(C=O); 192.78(C=O); Analysis: Calc. for $C_{23}H_{18}N_4O_4$: C, 66.66; H, 4.38; N, 13.52; O, 15.44; Found: C, 66.62; H, 4.36; N, 13.56; O, 15.47.

4.2.3. Ethyl [3-(2-ethoxy-2-oxoethyl)-2-imino-5-benzoyl-2,3-dihydro-1H-benzimdazol-1-yl]acetate **12:** IR, KBr, (cm^{-1}): νNH 3457; νC=O 1736; νC=O 1691 δNH 1649; δCH_3 1377; νC-O-C1208; δAr 746, 713; ^{1}H NMR (DMSO-d6) δ (ppm): 1.191 (t, J=7.1Hz, 6H, CH_3), 4.152 (q, J=7.1 Hz, 4H, OCH_2), 4.262 (s, 4H, NCH_2), 7.107 (d, J=8.1Hz, 1H, 7-Ar), 7.346(d, J=8.1Hz, 1H, 6-Ar), 7.446 (s, 1H, 4-H), 7.536 (t, J=7.7Hz, 2H, m-Ph), 7.640 (t, J=7.4Hz, 1H, p-Ph), 7.678(dd, J=8.2, 1.2Hz, 2H, o-Ph); ^{13}C NMR (DMSO-d6) δ (ppm): 13.97 (CH_3), 47.48 (NCH_2), 60.14 (OCH_2), 105.77 (7-C), 107.92 (4-Ar), 109.58 (6-Ar), 128.37 (m-Ph), 129.10 (7a-C), 129.30 (o-Ph), 131.95 (p-Ph), 132.25

(3a-C), 138.06 (i-Ph), 152.22 (C=N), 167.77 (O-C=O), 171.52 (C=O). Analysis: Calc. for $C_{22}H_{23}N_3O_5$; C, 64.54; H, 5.66; N, 10.26; O, 19.54; Found: C, 64.57; H, 5.61; N, 10.28; O, 19.49.

4.2.4. Ethyl [3-(2-ethoxy-2-oxoethyl)-2-imino-5-methyl-2,3-dihydro-1H-benzimdazol-1-yl]acetate hydrobromide **13:** Yield – 65 %; Mp 297-299 °C, Rf – 0,66; mobile phase $CHCl_3$/ $CH_3COOC_2H_5$; IR, KBr, (cm^{-1}): νNH 3342; νC=O 1733; δNH 1666; δCH_3 1378; ^{1}H NMR (DMSO-d6) δ (ppm): 1.226 (t, 6H, $2CH_3$), 2.389 (s, 3H, CH_3), 4.207 (q, 4H, $2O\text{-}CH_2$), 5.168 (s, 4H, $2CH_2$), 7.154 (d, 1H, Ar), 7.461 (t, 2H, Bz), 9.039 (bs, 2H, NH_2^+); ^{13}C-NMR(DMSO-d6) δ (ppm): 13.53 (C-20), 14.08 (C-23), 21.03 (C-17), 61.85 (C-19, C-22), 110 (C-9), 127.69 (C-6), 133.87 (C-2), 150.65 (C=N), 166.50 (C=O); Analysis: Calc. for $C_{16}H_{21}N_3O_4$: C, 60.17; H, 6.63; N, 13.16; O, 20.04; Found: C, 60.20; H, 6.61; N, 13.14; O, 20.06.

6.2.5. 5-Methyl-1,3-bis(3-phenylpropyl)-1H-benzo[d]imidazol-2(3H)-imine hydrobromide ***14***:

Yield - 72 %, Mp 223-225 °C, Rf- 0.55, mobile phase CH_2Cl_2/CH_3OH**;** IR, KBr, (cm^{-1}): νNH-3409; δNH 1676; δCH_3 1382; ^{1}H NMR (DMSO-d6) δ (ppm): 1.987 (t, 4H, $2CH_2$); 2.399 (s, 3H, CH_3); 2.643 (m, 4H, $2CH_2$); 3.467 (m, 4H, $2CH_2$); 7.168 (m, 7H, CH); 7.249 (m, 4H, CH); 7.314 (s, 1H, CH); 8.738 (s, 2H, NH_2^+); Analysis: Calc. for $C_{26}H_{29}N_3$: C, 81.42; H, 7.62; N, 10.96; Found: C, 81.42; H, 7.62; N, 10.96.

4.3. Synthesis of (5-phenylcarbonyl-1H-benzimidazol-2-ylthio)acetic acid ***15****:* A solution of sodium hydroxide (0.012 mol) in ethanol (14 mL) and 5(6)-(un)substituted-1H-benzimidazole-2-thiol (0.0067 mol) was refluxed for 1 h. After cooling, chloroacetic acid (0.0067 mol) was added and the refluxing was continued for 5 h more. Then the reaction mixture was cooled, poured into water and acidified with diluted acetic acid. The crystallized product was filtrated, carefully washed with water and re-crystallized with ethanol; Yield: 85%; Mp 173-175; Rf 0.4 C_6H_6/CH_3OH = 2:1; IR, KBr, (cm^{-1}): νOH- 3440; νNH 3208; νC=O 1747; νC=O 1649; ^{1}H NMR (DMSO-d6) δ (ppm): 3.91 (s, 2H, CH2); 7.58 (dd, 2H, m-Ph, J= 7.15 Hz); 7.70 (m, 1H p-Ph); 7.87 (d 1H, o-Bz, J=8.40 Hz); 7.92 (d, 1H, o-Ph, J=7.38 Hz); 7.94 (m, 1H, o-Bz); 8.04 (d, 1H, m-Bz); 9.82 (bs, 2H, NH, COOH);

Analysis: Calc. for $C_{16}H_{12}N_2O_3S$; C, 61.53; H, 3.87; N, 8.97; O, 15.37; S, 10.27; Found: , 61.55; H, 3.89; N, 8.95; O, 15.34; S, 10.29.

4.4. Synthesis of 6-(phenylcarbonyl)[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one **16***:* A solution of 5-phenylcarbonyl-(1H-benzimidazol-2-ylthio)acetic acid (0.104 mol) and acetic anhydride (19.5 mL) in dry pyridine (65 mL) was heated under reflux for 10 min. After completing of the reaction the solution was cooled and the product was crystallized as pale orange sediment by addition of water (100 mL) in small portions by stirring. Yield 66%; Mp-147-150; °C, Rf 0.5; mobile phase $CH_3COOC_2H_5/C_6H_6$ - 1:4; IR, KBr, (cm^{-1}): νC=O 1747; νC=O 1648; νC=O 1648; νC-S 1369, 1293; ^{1}H NMR (DMSO-d6) δ (ppm): 4.648 (s, 2H) and 4.652 (s, 2H, CH_2), 7.55-7.60 (m, 4H, m-Ph), 7.69-7.72 (m, 2H, p-Ph+p-Ph), 7.73-7.76 (m, 6H, o-Ph+7-Ar+8-Ar), 7.806 (dd, J=8.4 Hz, J=1.7 Hz, 1H, 7-Ar), 7.894(d, J=1.1 Hz, 1H, 5-Ar), 8.014(d, J=8.2 Hz, 1H, 8-Ar), 8.157(d, J=1.2 Hz, 1H, 5-Ar); Analysis: Calc. for $C_{16}H_{10}N_2O_2S$; C, 65.29; H, 3.42; N, 9.52; O, 10.87; S, 10.89; Found: $C_{16}H_{10}N_2O_2S$; C, 65.31; H, 3.40; N, 9.54; O, 10.88; S, 10.85.

4.5. General procedure for compounds **17-20**

A) 0.0105 mol of the relevant aldehyde was added to the solution of 0.01 mol 3a-d in 50 mL absolute ethanoland 3-4 drops of pyridine. After several hours of reflux, the solution was cooled and the precipitate obtained was washed with ethanol, followed by double re-crystallization from ethanol.

B) Mixture of 0.01 mol 1H-benzimidazole-2-thiol, 0.015 mol chloroacetic acid, 0.01 mol aromatic aldehyde, 2-3 drops piperidine and glacial acetic acid were refluxed for 4 h. After cooling, the product obtained was re-crystallized with ethanol.

4.5.1. 2-(4-fluorobenzylidene)-6-(phenylcarbonyl)[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one ***17:***

Yield: 66 /76 %.; Mp 209-210 °C; Rf = 0.3, mobile phase: $CH_3COOC_2H_5/C_6H_6$ - 1: 8; IR, KBr, (cm^{-1}): νC=O 1721; νNO_2 1341; δAr 829; ^{1}H NMR ($CDCl_3$): 8.505 (s, 1H, CH), 8.350 (ds, 1H, CH),

8.146 (t, 1H, 1CH), 7.904 (q, 1H, 1CH), 7.849 (d, 2H); 7.783 (d, 1H), 7.672-7.612 (dt, 2H), 7.515 (q, 3H, 3CH), 7.353 (t, 1H, CH); Analysis: Calc. for $C_{23}H_{13}FN_2O_2S$: C, 68.99; H, 3.27; F, 4.74; N, 7.00; O, 7.99; S, 8.01; Found: C, 68.95; H, 3.31; F, 4.73; N, 7.04; O, 7.94; S, 8.04;

4.5.2. 2-(2-fluorobenzylidene)-6-(phenylcarbonyl)[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one **18:** Yield - 51/65 %; Mp -232-235°C; Rf 0.64, mobile phase: $CH_3COOC_2H_5/C_6H_6$ - 1: 8; IR, KBr, (cm^{-1}): νC=O 1732; νC=O 1653; νAr 1613; 1550; 1506; δAr 756; 698; ^{1}H NMR ($CDCl_3$): 8.504 (s, 1H, CH), 8.077 (m, 1H, CH), 7.917 (d, 1H, 1CH), 7.888 (t, 1H, 1CH), 7.806 (d, 2H); 7.774 (d, 1H), 7.648 (m, 3H), 7.537 (m 3H); Analysis: Calc. for $C_{23}H1_3FN_2O_2S$: C, 68.99; H, 3.27; F, 4.74; N, 7.00; O, 7.99; S, 8.01; Found: C, 68.91; H, 3.27; F, 4.76; N, 7.06; O, 7.96; S, 8.05;

4.5.3. 2-(2-methoxybenzylidene)-6-(phenylcarbonyl)[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one **19** Yield- 62/75 %; Mp - 235-237°C; Rf 0.62, mobile phase: $CH_3COOC_2H_5/C_6H_6$ - 1: 8; IR, KBr, (cm^{-1}): νAr 3031; νCH_3 2840; νC=O 1725; νC=O 1654; νAr 1596; 1560; 1487; δCH_3 1440; 1362; νC-O-C 1252; 1158; δAr 694, 746; δAr 722; ^{1}H NMR ($CDCl_3$): 8.500 (t, 1H, CH, J = 8.52Hz), 8.144(t, 1H, CH), 7,907 (m, 1H), 7.880 (d, 2H, 2CH); 7.769 (d, 1H, CH), 7.607 (m, 5H, 5CH); 7.116 (t, 1H, CH), 7.008 (d, 1H, CH), 3.958 (s, 3H, CH3); Analysis: Calc. for $C_{24}H_{16}N_2O_3S$: C, 69.89; H, 3.91; N, 6.79; O, 11.64; S, 7.77; Found: C, 69.91; H, 3.89; N, 6.77; O, 11.67; S, 7.74.

4.5.4. 2-(1,3-benzodioxol-5-ylmethylidene)-6-(phenylcarbonyl)-[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-one **20**: Yield: 45 /60 %; Mp - 165-167 °C; Rf 0.60 mobile phase $CH_3COOC_2H_5/C_6H_6$ = 1:4; IR, KBr, (cm^{-1}): νC=O 1717; νC=O 1654 ; νAr 1614; 1580; 1508; δCH_2 1440; νC-O-C 1244; 1112; ^{1}H NMR ($CDCl_3$) δ (ppm): 5.93 (s, 2H, CH2); 7.24 (dd, 2H, o-Pip, J = 8.22 Hz); 7.36-7.39 (m, 1H, m-Pip); 7.57 – 7.60 (m, 2H, o-Ph); 7.67 – 7.71 (m, 1H, p-Ph); 7.92 (m, 2H, o-Ph, J = 7.38 Hz); 7.99 (s, 1H, CH=C); 8.20 (d, 1H, o-Bz, J= 8.40 Hz); 8.36 (d, 1H, m-Bz); 8.47 (s, 1H, o-Bz); Analysis: Calc. for $C_{24}H_{14}N_2O_4S$: C, 67.60; H, 3.31; N, 6.57; O, 15.01; S, 7.52; Found: C, 67.62; H, 3.34; N, 6.54; O, 15.03; S, 7.49.

4.6. Assay for in vitro antibacterial activity

The *in vitro* antimicrobial activity of samples **10-14** and **17-20** was tested against a panel of laboratory control strains belonging to American Type Culture Collection Maryland, USA and National Collection of Type Cultures, UK. Antimicrobial activity was evaluated against two Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017).

The minimal inhibitory concentration (MIC) of samples, against tested bacteria was determined by using a broth microdilution method in 96 multi-well microtitre plates.[43] After overnight cultivation, microbial suspensions were made in Mueller Hinton broth and their turbidity was standardised to 0.5 McFarland. Dimethyl sulphoxide (10%, v/v aqueous solution) was used to dissolve and to dilute sample. A serial double dilution of the sample was prepared in 96 well microtitre plates, using method of Sarker et al.[44] The lowest concentration of the sample that inhibited visible growth was taken as the MIC value. One row was used as a positive control and contained a broad-spectrum antibiotic (doxycycline in a serial dilution of 200–0.05 μg/ml) to determine the sensitivity of Gram-negative and Gram-positive bacteria while the other row contained the solvent as negative control. Tests were carried out in triplicate.

4.7. Lipid peroxidation inhibition

Phospholipids (Phospholipon® 90; PL90) were gifted by PHOSPHOLIPID GMBH, Cologne, Germany. According to the accompanied declaration the mixture content of PL90 is: phosphatidylcholine 98%, lyso-phosphatidylcholine 2.1%; fatty acid composition: palmitic acid 12±2%, stearic acid 3±1%, oleic acid 10±3%, linoleic acid 66±5%, linolenic acid 5±2%, peroxide value max. 1.3. The PL90 were kept in dark to prevent at least the photooxidation process.

Thiobarbituric acid (TBA), 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) and standards of trolox, quercetin, caffeic acid and L-ascorbic acid were obtained from Sigma Aldrich.

4.7.1. TBA-MDA test

The reaction mixture of 0.3 mL contained methanol solution of lipids (PL90) ($1 \cdot 10^{-2}$ mol/L) and methanol solutions of selected synthesized compounds (1 g/L) in 2:1 (v/v) ratio. Lipid peroxidation was initiated by using 0.2 mL ($2.2 \cdot 10^{-2}$ mol/L) aqueous solution of hydrophilic thermal initiator of lipid peroxidation (AAPH – 2,2′-azobis(2-methylpropionamidine) dihydrochloride) during time period of 3 h at 40 °C. One centimeter cube of aqueous trichloroacetic acid (5.5 %), followed by 0.5 mL of TBA ($4.2 \cdot 10^{-2}$ mol/L in $5 \cdot 10^{-2}$ mol/L NaOH) and BHT ($1 \cdot 10^{-3}$ mol/L) were added in the reaction mixture immediately after 3 h of initiation. The mixture was incubated for 10 min at 65 ºC in the dark, and centrifuged for 5 min at 13800 rpm. The TBA-MDA complex absorbances in the supernatant (the absorbances of control, sample and blank solution monitoring MDA level in the lipid before LP initiation by AAPH) read at 530 nm were used to calculate the inhibition percentage of lipid peroxidation.[26]

Inhibition of Lipid Peroxidation (%) = 100•(Ac-As)/(Ac-Ab)

Ac – the absorbance of control, *As* – the absorbance of sample and *Ab* – the absorbance of blank solution monitoring MDA level in the lipid before LP initiation by AAPH.

All samples were assayed for LP inhibitory activity at concentrations of 200 µg/mL in the fiinal reaction mixture. Those showing inhibition greater than 50% at this concentration were tested in a broader concentration range to allow calculation of IC_{50} values.

4.8. Computational details

All theoretical calculations were performed using the Gaussian 09 package[45] of programs. Geometry and vibrational frequencies of the studied species were performed by analytical gradient technique without any symmetry constraint. All the results were obtained using the density

functional theory (DFT), employing the B3LYP (Becke's three-parameter non-local exchange[46] and Lee et al. correlation[45] potentials).

The geometries of all possible isomers of the studied compounds, radicals, radical cations were fully optimized by application of the UB3LYP functional in conjunction with the 6-311++G** basis set. The optimized structures were further characterized by analytical computations of harmonic vibrational frequencies at the same level. Dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) were calculated according the equations given by Klein et al.[27]

$BDE = H(R^{\cdot}) + H(H^{\cdot}) - H(R\text{-}H)$

$IP = H(R^{+\cdot}) + H(e^{-}) - H(R\text{-}H)$

$PDE = H(R^{\cdot}) + H(H^{+}) - H(R^{+\cdot})$

$PA = H(R^{-}) + H(H^{+}) - H(R\text{-}H)$

$ETE = H(R^{\cdot}) + H(e^{-}) - H(R^{-})$

The enthalpy of hydrogen atom, $H(H)$ was obtained by the same method and basis set. All reaction enthalpies were calculated at 298 K. The enthalpies of proton $H(H^{+})$, and electron, $H(e^{-})$, were taken from the literature: 6.197 kJ/mol and 3.145 kJ/mol, respectively.[27] Natural bond orbitals (NBO) analysis[47-49] has been performed to characterize the delocalization of electron density within the molecule.

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List of captions

Figure 1. Hydrogen atom abstraction at different sites and corresponding DFT B3LYP/6-311++G** bond dissociation enthalpies of **12** in kJ/mol.

Figure 2. Hydrogen atom abstraction and corresponding DFT B3LYP/6-311++G** bond dissociation enthalpy of **17** in kJ/mol.

Figure 3. Stereo isomers of the methoxy adduct of **17** – S configuration at C6 (A) and R configuration at C6 (B).

Scheme 1. Synthesis of the studied benzimidazole compounds. Reagents and conditions: a) $KMnO_4$, NaOH water solution, refluxing; b) NH_4OH, 150 °C in welded ampoule; c) TBAB, dry K_2CO_3, halogen derivative, 25 °C, acetonitrile; d) $ClCH_2COOH$, NaOH/ethanol, refluxing 3 h; e) $(CH_3CO)_2O$, pyridine medium refluxing 10 min; aromatic aldehydes, cat. Py, ethanol refluxing 4-6 h.

Scheme 2. Hypothetical mechanism of antioxidant action of compounds **10-14.**

Scheme 3. Hypothetical mechanism of antioxidant action of compounds **17-20.**

Scheme 4. Resonance structures of the peroxyl scavenger (radical cation) of **17.**

Table 1. Minimal inhibitory concentrations (MIC values given in mg/mL, unless stated otherwise) of compounds **10-14**

Table 2. Lipid peroxidation inhibition effects of studied benzimidazoles **10-14** and **17-20** (example concentration 200 μg/mL) and IC_{50} values (given in μg/mL)

Table 3. Calculated molecular properties of compounds **10-14** and **17-20** for assessment of the drug-likeness

Graphical abstract