

**Medicinal chemistry** 

# Hydroxyl Radical as Key Intermediate in Curing Action of Artemisinin and its Analogs

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## Abstract

A kinetic analysis of intramolecular oxidation reactions of 1 derivatives in combination with the published data on antimalarial activity makes it possible to formulate the following mechanism of action of the peroxide drugs, analogs of compound 1. Under the reaction of the Fe(II) chelates the compound containing the peroxide group is transformed into the alkoxyl radical. This radical isomerizes to the alkyl radical, which further undergoes intramolecular chain oxidation. This oxidation results in polyatomic hydroperoxides, which, in turn, generates radicals in the reaction with Fe(II). The next cascade of radical reactions generates very reactive hydroxyl radicals, whose sources are peroxyl radicals with hydroperoxide fragments and  $\alpha$ -dihydroperoxides. The higher the yield of hydroxyl radicals nOH, the more efficient the drug. The dependence of the antimalarial activity of the ith drug IC50(1)M(1)/IC50(i)M(1) on the yield of radicals HO nOH is nonlinear (exponential). The compounds with nOH  $\geq$  3 are efficient.

**Keywords:** Artemisinin; Derivatives; Free radicals; Hydroxyl; Oxidation; Hybrid; Isomerization

## Introduction

Artemisinin [1] is a highly efficient drug, different modifications of which are successfully used against malaria plasmodium (*Plasmodium falciparum*) resistant to quinine and its analogs of the alkaloid type. The exited history of opening and study of compound 1 is described in a line of reviews [1-13] Structure 1 is a sesquiterpene endoperoxide. The curing effect of compound 1 was proved to be result of generation of free radicals [1,2,5,14-20]. The peroxide bridge in structure 1 generates free radical *via* the redox reaction with chelates of divalent iron

ROOR +  $Fe^{2+} \rightarrow RO^{-} + Fe^{3+}$ , which, as it is believed, results in the death of the parasite. It was assumed for a long time that the mechanism of the antimalarial effect of compound 1 is reduced to this reaction, i.e., compound 1 "works" as a usual initiator [4-20]. However, the synthesis and testing of its analogs on antimalarial activity showed that the whole structure of molecule 1 rather than its peroxide bridge only plays an important role [1,2]. Using the kinetic analysis of the reactions occurring after the cleavage of the peroxide bridge in compound 1 in the presence of oxygen, it was proved that the free radicals formed are involved in a cascade of consecutive transformations with participation of oxygen [21-27]. As a result, compound 1 is transformed into polyatomic hydroperoxide. The latter generates a line of free radicals via the reaction with Fe(II), which results in the death of the parasite. It was evidenced in our latest kinetic studies that namely hydroxyl radical, produced in some reactions of 1 oxidation plays the key role in curing action of 1 and its peroxide analogs [28-31]. This paper summarizes the results received during the latest four years on kinetic analysis of reactions induced by 1 and its derivatives.



## Intramolecular Oxidation of Artemisinin

Under aerobic conditions in the living organism, cycloalkyl radicals derived from 1 add oxygen. The addition of O<sub>2</sub> to alkyl radicals is limited only by the self-diffusion of oxygen and occurs with a high rate constant ( $k_{o2}$ ) of 10<sup>9</sup>  $\div$  10<sup>10</sup> liter mol<sup>-1</sup> s<sup>-1</sup>. In lipids, the constant  $k_{o2}$  is similar to those in linoleic acid esters where it is equal to  $k_{o2}$  =1.56  $\times$  10<sup>9</sup> liter mol<sup>-1</sup> s<sup>-1</sup> [32]. The Henry coefficient of oxygen ( $\gamma_{o2}$ ) in this medium is  $\gamma_{o2}$  = 1.12  $\times$  10<sup>-2</sup> mol liter<sup>-1</sup> atm<sup>-1</sup> [32]. Hence, the specific rate of the reaction of alkyl radicals with oxygen at p(O<sub>2</sub>) = 0.21 atm (in air) is equal to  $k_{o2}\gamma_{o2}p(O_2)$  = 3.56  $\times$  10<sup>6</sup> s<sup>-1</sup>. A comparison of the oxygen addition with the monomolecular reactions of the artemisinin alkyl radicals as well as the reaction with L-cysteine showed that this reaction is the most rapid transformation channel for the majority of radicals [26,27]. Therefore, under aerobic conditions these are the following main reaction channels of radicals generated from peroxide bridge of artemisinin:



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The further transformations of the artemisinin peroxyl radicals were analyzed for the first time in the studies [24,25]. The formed peroxyl radical induces in **1** a cascade of intramolecular chain reactions with production of artemisinin with a line of hydroperoxide groups. Down are given examples of such reactions [25].



When the possibility of intramolecular chain reaction inside **1** is exhausted, peroxyl radical reacts with any substrate. Among them thio-groups of L-cysteine are the most reactive (Figure 1), and free valence abandons the molecule in the form of thiyl radical [25].

Peroxyl radicals participate not only in consecutive, but parallel reactions too. There are given in Table 1, the parameters for all parallel reactions of radical C<sup>8</sup>OO<sup>•</sup>.

The formed hydroperoxide groups are decomposed by Fe(II) into alkoxyl radicals that, in its turn, start a new cascade of oxidation reactions. The kinetic scheme of 1 oxidation is given dawn with kinetic characteristics of every limiting stage.

Each stage involving the peroxyl radical includes two consecutive elementary acts: the fast addition of oxygen to the alkyl radical and the intrinsic fast reaction involving RO<sub>2</sub><sup>•</sup> formed. This scheme shows that alkoxyl radical RO<sup>•</sup>(1) formed from compound **1** isomerizes exclusively to alkyl radical R<sup>•</sup>(5a). The single parallel reaction of RO<sup>•</sup>(2) with LSH is by three orders of magnitude slower (k(LSH) =  $1.0 \times 10^6 \text{ s}^{-1}$ ). As a result of a chain of successive radical transformations, R<sup>•</sup>(5a) generates four hydroxyl radicals. Since only 50% of the molecules reacted with Fe(II) generates



Reaction	Δ <i>H</i> , kJ/mol	<i>E</i> , kJ/mol	<i>k</i> (310 К), s⁻¹	%
$C^{8a}OO \bullet \rightarrow C^{12} \bullet$	19.9	44.6	1.71 × 10⁵	62.6
$C^{8a}OO \bullet \rightarrow C^{8}(O) + HO \bullet$	-110.5	42.7	6.78 × 10 <sup>4</sup>	25.0
$C^{8a}OO \bullet \rightarrow C^{5a} \bullet$	31.7	50.3	1.81 × 10 <sup>4</sup>	6.4
$C^{8a}OO\bullet \rightarrow C^{10\bullet}$	34.4	51.7	1.07 × 10 <sup>4</sup>	4.0
$C^{8a}OO \bullet \rightarrow C^{6} \bullet$	38.2	53.6	5.12 × 10 <sup>3</sup>	1.8
C <sup>8a</sup> OO•+LSH→C <sup>12</sup> OOH +LS•	1.6	32.4	6.93 × 10 <sup>1</sup>	0.03

Table 1: Kinetic parameters of parallel reactions of  $C^{8a}$  peroxyl radical of 1.

radicals RO<sup>•</sup>(2), the yield of radicals HO<sup>•</sup> per molecule 1 *via* this radical is equal to 2. The transformation of radical RO<sup>•</sup>(1) proceeds *via* two parallel channels. The total yield of radicals  $(n_{\Sigma R})$  per one molecule of 1 is  $n_{\Sigma R} = 4.34$ , of which  $n_{OH} = 3.17$  and  $n_{LS} = 1.17$ .

One can suppose that the more free radicals are produced by one molecule of drug curing by free radical generation, the more will be activity of antimalarial drug. A kinetic scheme of oxidation was built up for a line of 1 derivatives with the following structures [28-30]:



The total yield of free radicals  $n_{\Sigma R}$  was compared with values of relative indexes IC<sub>50</sub> (Figure 2) [28-31].

The result appeared paradoxal: it is clearly seen that any correlation is absent. On the one hand, there are compounds with  $n_{\Sigma R}$  equal to 3.5 and 5 among highly efficient antimalarial drugs. On the other hand, the compound with  $n_{\Sigma R} = 6$  turned out lowly efficient. Therefore, the high total yield of free radicals does not indicate that the drug is efficient.

# The Important role of Hydroxyl Radicals

It is seen from scheme 1 that free radicals  $\mathbb{R}^{\bullet}$ ,  $\mathbb{RO}^{\bullet}$ , and  $\mathbb{RO}_{2}^{\bullet}$ , formed in the process of 1 oxidation, are transformed inside the oxidized molecule. Free valence abandons the molecule in the forms of thiyl or hydroxyl radicals. Thiyl radicals are formed in bimolecular reactions of  $\mathbb{RO}^{\bullet}$  or  $\mathbb{RO}_{2}^{\bullet}$  radicals with thio-groups of L-cystein in polypeptides. They have low activity and recombine with resulting scission of polypeptides [32-37].



Figure 2: Correlation between logarithm of relative antimalarial activity In {IC<sub>50</sub>(1)/IC<sub>50</sub>(i)} of artemisinin derivatives 1-13 and total number of free radicals  $\Sigma R$  produced in the result of their oxidation.







Scheme 1: The mechanism of 1 oxidation; the values of enthalpies ( $\Delta H$ , kJ/mol), activation energies (E, kJ/mol) and rate constants ( $k(310 \text{ K}) \text{ s}^{-1}$ ) were calculated by the method of intersecting parabolas: [32-34].

 $RO_2$  + LSH  $\rightarrow$  ROOH + LS<sup>•</sup>

 $RO^{\bullet} + LSH \rightarrow ROH + LS^{\bullet}$ 

 $\mathsf{LS}^{{\scriptscriptstyle\bullet}} + \mathsf{LS}^{{\scriptscriptstyle\bullet}} \to \mathsf{LSSL}$ 

Hydroxyl radicals are produced by isomerization of  $RO_2^{\phantom{1}}$  of the type:

 $RCH(OO^{\bullet})CH_{,}CH(OOH)R \rightarrow RCH(OOH)CH_{,}C(O)R + HO^{\bullet}$ 

and in the result of decomposition of  $\alpha$ -dihydroperoxide:

 $\text{RCH}(\text{OOH})\text{CH}(\text{OOH})\text{R} + \text{Fe}(\text{II}) \rightarrow \text{RCH}(\text{O}^{\bullet})\text{CH}(\text{OOH})\text{R} + \text{Fe}(\text{III}) + \text{HO}^{-}$ 

 $RCH(O)CH(OOH)R \rightarrow RCH(O) + RCH(O) + HO$ .

Reactions of hydroxyl generation are extremely exothermic. For example, the reaction



proceeds with enthalpy  $\Delta H = -126$  kJ/mol. Very exothermic is the reaction of decomposition of  $\alpha$ -hydroperoxyalkoxyl radical formed from  $\alpha$ -dihydroperoxide. For example, the reaction



proceeds with  $\Delta H = -137$  kJ/mol.

The hydroxyl radicals due to high energy of formed O–H bond ( $\approx 500 \text{ kJ/mol}$ ) are extremely active in reactions of hydrogen atom abstraction. And we observe quite another situation when we compare IC<sub>50</sub> with the relative yield of hydroxyl radicals by **1** derivatives (Table 2).

The results of comparison of  $\ln\{M(i)IC_{50}(1)/IC_{50}(i)M(1)\}$  and  $n_{OH}$  are presented on (Figure 3). They indicate an explicit linear dependence between these values with a correlation coefficient of 0.97 and the root-mean-square deviation SD = 0.5 inside interval of  $n_{OH}$  = 2.5 ÷ 4.0.

This means that the curing effect of 1 derivatives is caused, first of all, by the *generation of hydroxyl radicals* that destroy malaria plasmodium. The dependence of  $\ln\{M(i)IC_{50}(1)/M(1)IC_{50}(i)\}$  on the yield of hydroxyl radicals is expressed by the following equation:

 $\ln\{M(\mathbf{i})\mathrm{IC}_{50}(\mathbf{1})/M(\mathbf{1})\mathrm{IC}_{50}(\mathbf{i})\} = 3.9(n_{\mathrm{OH}}(\mathbf{i}) - 3.17)$ 

(1)

Drugs that are more active than compound 1 should provide the yield of hydroxyl radicals exceeding 3.17. The kinetic study of oxidation of dimers of 1 proved, however, that this dependence changes its character. When  $n_{OH}(i)$  of compound becomes more than 4, the dependence has the following form [38]:

Compound	n <sub>он</sub>	Ν <sub>ΣR</sub>	/C <sub>50</sub> (1)//C <sub>50</sub> (i)	In[IC <sub>50</sub> (1)/IC <sub>50</sub> (i)]	Ref.
1	3.17	4.00	1.00	0.00	35
2	4.00	4.50	7.40	2.00	35
3	3.83	4.66	2.28	0.82	35
4	3.83	4.66	1.78	0.58	35
5	1.67	4.33	0.08	-2.53	35
6	3.00	5.00	0.20	-1.61	36
7	3.30	4.30	1.08; 0.75 *	0.08; -0.29	36
8	3.00	6.00	0.14	-1.97	36
9	0.50	1.00	0.00; 0.00 *	-	36
10	2.83	4.50	0.09	-2.41	37
11	3.50	4.00	2.20; 1.80; 1.40 *	0.79; 0.59; 0.34	37
12	2.50	3.00	0.016	-4.13	37
13	2.50	3.00	0.016	-4.13	37

**Table 2:** Comparison of the antimalarial activity of compound 1 and its analogs (2–13) [35-37] with the number of hydroxyl radicals  $n_{OH}$ , which are formed due to their intramolecular oxidation [28-31].



# $M(\mathbf{i})$ IC<sub>50</sub>(1)/ $M(\mathbf{1})$ IC<sub>50</sub>( $\mathbf{i}$ ) = 1 + 0.27( $n_{\text{OH}}(\mathbf{i})$ - 3.17)

(2)

Why do precisely the hydroxyl radicals cause the death of the malaria parasite? They are very reactive due to the high exothermicity of radical abstraction reactions involving these radicals. This is seen from the comparison of the O–H bond strength in various compounds [39].

Compound / (D<sub>0-H</sub>, kJ mol<sup>-1</sup>) sec-ROOH(365.5), PhO-H(369.0), MeO-H(436.0), HO-H(499.0)

For this reason, hydroxyl radical reacts with many reactants with a huge rate constant and is poorly selective. The reaction of hydroxyl addition is also very fast. In particular, it reacts with organic bases composing DNA with diffusion rate constant. The values of  $k(HO^{\bullet} + base)$  in H<sub>2</sub>O are presented below [40].

Base / (k × 10<sup>-9</sup> / l mol<sup>-1</sup> s<sup>-1</sup>, 298 K) Adenine (5.5, pH = 5.7), Guanine (5.8, pH = 10.0), Thymine (5.8, pH = 6.0), Cytosine (9.2, pH = 7.0)

So, it is likely that it is the DNA of malaria plasmodium that is the biological target, whose reaction with HO' radicals results in the death of the parasite. It was experimentally shown that in the presence of iron ions of compound **1** caused the destruction of DNA plasmodium [1,41,42]. Another biological target of hydroxyl radical may be catalytic Fe-centers of enzymes that plays important role in parasite life [6].

# Cyclohexyl Endoperoxides

The cyclohexyl endoperoxides are known as potential antimalarials. The following compounds **14-28** were sinthesised and tested on antimalarial activity by M. P. Crespo et al. [43].



The kinetic scheme of free radical reactions of endoperoxides 14-17 is presented below.

Since the substituents R in the third and sixth positions are uninvolved in the free radical reactions, this scheme is the same for all of the four compounds (14–17). Alkoxyl radicals result from the decomposition of an endoperoxide by a Fe(II) chelate. They isomerizes rapidly into alkyl radicals, and the latter turn rapidly into peroxyl radicals in the presence of oxygen [32]. All parallel reactions were considered for each reaction step, and the most rapid of them are included in Scheme 2. In the cases in which the rate constants of two parallel reactions are comparable (their ratio is no larger than 5), were taken into account. The results of comparison of the molar effectiveness of the endoperoxides  $M(i)IC_{50}(1)/M(1)IC_{50}(i)$  with total yield of free radicals  $n_{yR}$  are presented in (Table 3).

Clearly, peroxides characterized by the same free radical yield differ in their antimalarial effectiveness. Averaging of the  $M(1)IC_{50}(i)/M(i)IC_{50}(1)$  values yielded the following results:

Endoperoxides	14-17	18-26	27-28
n <sub>zR</sub>	1.0	2.0	2.68
M(1)IC <sub>50</sub> (i)/ $M(i)$ IC <sub>50</sub> (1)	$2.3  imes 10^{-3}$	$2.3 \times 10^{-2}$	$3.3 \times 10^{-2}$

The antimalarial effectiveness of the peroxides depends linearly on the total radical yield  $n_{y_R}$  and can be fitted to the linear equation (3).

 $IC_{50}(1)M(i)/IC_{50}(i)M(1) = (1.85 \pm 0.16) \times 10^{-2} (n_{yp} - 0.84)$ 

Thus, the antimalarial activity of the monocyclic endoperoxides increases with an increasing total free radical yield per unit amount of peroxide

(3)



N	n <sub>HO</sub>	n <sub>HO2</sub>	n <sub>LS</sub>	n <sub>zR</sub>	M(1)IC <sub>50</sub> (i)/M(i)IC <sub>50</sub> (1)	
					Clone D10	Clone K1
14	1.00	0.45	1.24	2.69	2.1 × 10 <sup>-2</sup>	4.4 × 10 <sup>-2</sup>
15	1.00	0.45	1.24	2.69	1.7 × 10⁻²	-
16	1.00	0.45	1.24	2.69	4.8 × 10 <sup>-2</sup>	5.1 × 10 <sup>-2</sup>
17	1.00	0.45	1.24	2.69	1.9 × 10⁻³	-
18	0	0	2	2.00	2.9 × 10 <sup>-2</sup>	6.0 × 10 <sup>-2</sup>
19	0	0	2	2.00	5.7 × 10⁻²	3.1 × 10⁻²
20	0	0	2	2.00	1.5 × 10⁻¹	1.6 × 10⁻¹
21	0	0	2	2.00	7.3 × 10⁻³	-
22	0	0	2	2.00	1.1 × 10 <sup>-2</sup>	1.9 × 10 <sup>-2</sup>
23	0	0	2	2.00	7.2 × 10⁻³	-
24	0	0	2	2.00	4.0 × 10 <sup>-2</sup>	7.7 × 10 <sup>-2</sup>
25	0	0	2	2.00	1.5 × 10⁻³	-
26	0	0	2	2.00	1.1 10 <sup>-3</sup>	8.4 × 10⁻³
27	0	0.40	0.60	1.00	2.4 × 10 <sup>-3</sup>	-
28	0	0.40	0.60	1.00	2.2 × 10⁻³	-

**Table 3:** Comparison of the antimalarial activity of endoperoxides **14-28**  $(M(1)|C_{50}(i)/M(i)|C_{50}(1))$  [43] with the number of free radicals, which are formed due to their intramolecular oxidation [44].

decomposed. The  $IC_{50}(1)M(i)/IC_{50}(i)M(1)$  ratio is a linear function of the total free radical yield. The peroxides characterized by  $n\Sigma R \le 1$  show almost no antimalarial activity. The above empirical relationship between the antimalarial effectiveness and  $n_{\Sigma R}$  provides an explanation for the fact that the linear peroxides ROOR and ROOH are inactive against malaria [1]. The activity of the monocyclic endoperoxides is only 0.2–3% of the activity of **1**. The results reported here will augment insight into the correlation between free radical generation and the antimalarial action of peroxide drugs. We will now compare the structural and kinetic characteristics of monocyclic peroxide drugs to those of polycyclic ones, to which artemisinin derivatives belong.

Cyclohexyl endoperoxides (14-28)	Artemisinin and its derivatives (1-13)
Monocyclic structure.	Polycyclic structure.
Domination of the RO <sub>2</sub> + LSH $\rightarrow$ ROOH + LS <sup>•</sup> reaction because of the	Domination of the RO <sub>2</sub> $\rightarrow$ R <sup>•</sup> reaction because of the high reaction rate
low rate of the $RO_2 \rightarrow R^{\bullet}$ reaction in the linear radical.	in the cyclic radical.
Short intramolecular oxidation chain (1 or 2 steps).	Long intramolecular oxidation chain (2-6 steps).
Free radical yield of 1 to 3.	Free radical yield of up to 6.
One or 2 OOH groups formed.	Up to six OOH groups formed.
HO• yield of 0 to 1.	HO• yield of 2 to 4.
Linear dependence of IC <sub>50</sub> on $n_{\Sigma R}$ .	Exponential dependence of $IC_{50}$ on $n_{OH}$ .
Relative antimalarial activity of 0–3%.	Relative antimalarial activity of 20-200%.

Clearly, the great difference in initiation behavior and therapeutic action between the monocyclic and polycyclic peroxides is due to the fact that the radicals of the former undergo comparatively slow intramolecular oxidation, while the radicals of the latter do this rapidly. The isomerization reaction R<sup>•</sup> in the cyclic structure is accompanied by a slight decrease in entropy and, as a consequence, is characterized by a large pre-exponential factor of  $A = 1.4 \times 10^{12} \text{ s}^{-1}$ . Monocyclic alkoxyl radical often decompose to yield linear radicals. The radicals forming from the latter isomerize at a low rate because of the high negative activation entropy (for these reactions,  $A = 2.0 \times 10^9 \text{ s}^{-1}$ ). As a consequence, the linear peroxyl radicals more readily enter into the bimolecular reaction:

 $RO_{2}$  + LSH  $\rightarrow$  ROOH + LS<sup>•</sup>,

so the peroxide does not turn into a polyatomic hydroperoxide, and this has adverse implications for its therapeutic action. The results of this study supplement earlier data concerning free radicals of 1 and its derivatives. The antimalarial activity of the endoperoxides is mainly determined by two factors. The major factor is generation of hydroxyl radicals by the polyatomic hydroperoxides that result from the chain intramolecular oxidation of the drug. The higher the HO<sup>•</sup> yield, the higher the antimalarial activity of the compound. The IC<sub>50</sub> value depends on  $n_{OH}$  in an exponential way (Equation 1). The other, less significant factor is generation of other radicals (RO<sup>•</sup>), which also kill the plasmodium, but do this less effectively. In terms of IC<sub>50</sub>, these radicals are 1–2 orders of magnitude less effective than the hydroxyl radicals. The IC<sub>50</sub> value depends linearly on  $n_{xR}$ . The peroxides generating a single radical are practically inactive against the plasmodium.

# Dispiro-1,2,4-trioxolanes

Some of dispiro-1,2,4-trioxolanes with various structures show high antimalarial activity, while others are less active [35,44,45]. A kinetic analysis of their radical transformations seems important for theoretically describing the therapeutic action of this new class of antimalarial drugs. This analysis was undertaken for to find answers to the following questions [46]. Is the therapeutic action of dispiro-1,2,4-trioxolanes related to their ability to initiate radicals? What is the role of hydroxyl radicals in this initiation? What is the relationship between the initiating ability of 1,2,4-trioxolanes and their structure? The structure of 7 dispiro-1,2,4-trioxolanes, whose radical transformations were analyzed [35,45], are given below:



The scheme of free radical reactions of endoperoxides 29-32 oxidation (the same for all these compounds) is presented below.

As was noted earlier, for 1 and its derivatives, hydroxyl radicals were shown to be most active. This also follows from a comparison of the molar antimalarial activity of dispiro-1,2,4-trioxolanes considered here with the yield of hydroxyl radicals  $n_{OH}$  (Table 4).

Drugs 29-32 generate more hydroxyl radicals than 1 does and are therefore more active. Trioxolane 33 generates less hydroxyl radicals than 1 does (2.58 vs 3.17), and its antimalarial index being smaller (0.3) than that of 1. Trioxolanes 34 and 35 generate only thiyl radicals, and therefore

Compound	n <sub>он</sub>	n <sub>Ls</sub>	Σn	<i>M</i> (i)IC <sub>50</sub> (1)/ <i>M</i> (1)IC <sub>50</sub> (i)
1	3.30	0.17	3.47	1.0
29	3.41	1.35	4.76	1.5 (1.54)
30	3.41	1.35	4.76	3.3
31	3.41	1.35	4.76	2.2
32	3.41	1.35	4.76	1.6
33	2.58	1.84	4.42	0.3 (0.06)
34	0.0	2.0	2.0	0.02 (0.02)
35	0.0	2.0	2.0	0.02 (0.02)

Table 4: Comparison of the antimalarial activity of dispiro-1,2,4-trioxalanes 29–35 [35,45] with the number of free radicals, which are formed due to their intramolecular oxidation [46].

their antimalarial index being two orders of magnitude smaller than that of **1**. For **1** and its derivatives, the empirical relationship between  $IC_{50}$  and  $n_{OH}$  is exponential (see Equation 1). According to this equation,  $IC_{50}(1)M(i)/IC_{50}(i)M(1) = 2.52$  for  $n_{OH} = 3.41$ . This closely coincides with its mean value 2.15 ± 0.70 for trioxolanes **29–32**. Trioxolanes **34** and **35** have a weak antimalarial activity (Table 4). Our analysis of the transformations of endoperoxides showed that the generation of only thiyl radicals also led to the antimalarial activity of the peroxide drug and the empirical relationship between  $M(i)IC_{50}(1)/M(1)IC_{50}(i)$  and  $n_{LS}$  was linear (see Equation 3). For  $n_{LS} = 2$  (compounds **33** and **34**),  $IC_{50}(1)M(i)/IC_{50}(i)M(1) = 0.02$ , which coincides with the empirical results for dispiro-1,2,4-trioxolanes (Table 3). The mechanism of the therapeutical activity of dispiro-1,2,4-trioxolanes containing a peroxide bridge is thus similar to that of **1** and its derivatives. Among the radicals formed by the oxidation of dispiro-1,2,4-trioxolanes, only hydroxyl radicals exhibit a unique antimalarial activity. They are formed from dispiro-1,2,4-trioxolanes, which generate *cyclic alkyl radicals* during the decomposition of alkoxyl radicals. The oxidation of the former radical gives rise to secondary hydroperoxide groups, whose subsequent radical transformation generates hydroxyl radicals. This is possible only in the case of dispiro-1,2,4-trioxolanes with polycyclic substituents (compounds **29–32**). Decyclization of primary alkoxyl radicals formed from monocyclic dispiro-1,2,4-trioxolanes results in the transformation of the latter into linear peroxyl radicals. Isomerization of the latter, forming >CHOOH groups, becomes rather slow, the reaction of with the protein thio-groups being faster. The generation of hydroxyl radicals thus becomes impossible. The thiyl radicals formed by the reactions of RO<sup>•</sup> and with LSH also show antimalarial activity. This is explained b

## Hybrid Analogs of 10-dihydroartemisinin

Recently, synthesis and application of so called hybrid antimalarial agents are being developed, which comprise both the structure **1** with a peroxide bridge and fragments of the quininelike alkaloids [47]. However, when these agents are evaluated as hybrid ones an important aspect is omitted. When the structure of the compound of **3** type is changed, it has an effect on the course of radical reactions, and, as a consequence, on its antimalarial properties. Higher effectiveness of the compound of this type may result both from the additional pathway involving the introduced fragment into nonradical mechanism and from the higher outcome of hydroxyl radicals formed in its radical transformation. This is why the hybrid action of antimalarial substances of this type cannot be evaluated by simple comparison of its effectiveness with that of compounds **1** or **3**. The aim of the work [48] was to study the effect of additional residues incorporated into the molecule **3** on the ability to generate hydroxyl radicals and to evaluate its possible influence on the antimalarial substance by another (nonradical) mechanism. For this purpose a special approach was elaborated and the kinetic study of the radical transformations was accomplished for a series of eleven hybrid compounds, which were synthesized and tested for antimalarial activity earlier [47].





Kinetic schemes of oxidation of compounds 37-46 are given by the example of compound 37 (Scheme 4).

The analysis of correlation between the therapeutic effect of compound 1 and its analogues 2–13 and the yield of hydroxyl radicals showed that





by the method of intersecting parabolas [32-34].

the dependence of the IC<sub>50</sub> value on the  $n_{\text{OH}}$  parameter is exponential (Equation 1) within the interval of  $n_{\text{OH}}$  from 2.5 to 4.0. The therapeutic effect of the generated hydroxyl radicals alone can be estimated using (Equation 1) on the basis of data given in (Table 2).

The comparison of experimental and calculated values of  $IC_{50}$  for compounds **36–45** are presented on (Table 5). The last colon contains the values of relative activity of drug that is result of generation of HO<sup>•</sup> only. They were calculated from the yield of hydroxyl radicals via Equation 1.

From data of Table 5 one can see that only compound **40** demonstrates activity higher than caused by generation of hydroxyl radicals. The activity of others are more than **1**, but lower than induced by hydroxyl radicals only. Hence, the kinetic analysis of radical reactions, which result from the transformations of the hybrid antimalarial compounds, revealed the two ways of action, one being caused by the hydroxyl radicals alone and the other one, that does not involve radical generation.

Compound	<b>п</b> <sub>он</sub>	n <sub>Ls</sub>	IC <sub>50</sub> (i)/ <i>M</i> (i)	IC <sub>50</sub> (1) <i>M</i> (i)/ IC <sub>50</sub> (i) <i>M</i> (1)	{IC <sub>50</sub> (1) <i>M</i> (i)/ IC <sub>50</sub> (i) <i>M</i> (1)} <sub>OH</sub>
1	3.17	1.17	12.5	1.00	1.00
36	3.83	0.83	7.2	1.74	13.1
37	3.42	1.69	8.2	1.52	2.65
38	3.42	1.69	138.1	0.09	2.65
39	3.42	1.69	6.3	1.98	2.65
40	3.42	1.69	12.5	1.00	2.65
41	3.42	1.69	3.8	3.30	2.65
42	3.42	1.69	6.6	1.89	2.65
43	3.42	1.69	4.3	2.91	2.65
44	3.42	1.69	13.3	0.94	2.65
45	3.42	1.69	22.5	0.55	2.65
46	3.42	1.69	12.6	0.99	2.65

Table 5: Comparison of the antimalarial activity of compound 1 and hybrid derivatives of 10-dihydroartemisinin (36 - 46) [47] with the number of hydroxyl radicals n<sub>OH</sub>, which are formed due to their intramolecular oxidation [48].

## Conclusion

Thus, a kinetic analysis of intramolecular oxidation reactions of 1 derivatives in combination with the published data on antimalarial activity makes it possible to formulate the following mechanism of action of the peroxide drugs, analogs of compound 1. Under the reaction of the Fe(II) chelates the compound containing the peroxide group is transformed into the alkoxyl radical. This radical isomerizes to the alkyl radical, which further undergoes *intramolecular chain oxidation*. This oxidation results in polyatomic hydroperoxides, which, in turn, generates radicals in the reaction with Fe(II). The next cascade of radical reactions generates very reactive *hydroxyl radicals*, whose sources are peroxyl radicals with hydroperoxide fragments and  $\alpha$ -dihydroperoxides. The higher the yield of hydroxyl radicals  $n_{OH}$ , the more efficient the drug. The dependence of the antimalarial activity of the i<sup>th</sup> drug IC<sub>50</sub>(1)*M*(1)/IC<sub>50</sub>(i)*M*(1) on the yield of radicals HO<sup>•</sup>  $n_{OH}$  is nonlinear (exponential). The compounds with  $n_{OH} \ge 3$  are efficient.

The mechanism of the therapeutical activity of dispiro-1,2,4-trioxolanes containing a peroxide bridge is thus similar to that of **1** and its derivatives. Among the radicals formed by the oxidation of dispiro-1,2,4-trioxolanes, only hydroxyl radicals exhibit a unique antimalarial activity. They are formed from dispiro-1,2,4-trioxolanes, which generate cyclic alkyl radicals during the decomposition of alkoxyl radicals. The oxidation of the former radicals gives rise to secondary hydroperoxide groups, whose subsequent radical transformation generates *hydroxyl radicals*. This is possible only in the case of dispiro-1,2,4-trioxolanes with polycyclic substituents (compounds **29–32**). Decyclization of primary alkoxyl radicals formed from monocyclic dispiro-1,2,4-trioxolanes results in the transformation of the latter into linear peroxyl radicals that reacts faster with the protein thio-groups. The generation of hydroxyl radicals thus becomes impossible.

The results of study the cyclohexyl endoperoxides supplement earlier data concerning free radical conversions of 1 and its derivatives. The antimalarial activity of the cyclohexyl endoperoxides is mainly determined by two factors. The major factor is generation of *hydroxyl radicals* by the polyatomic hydroperoxides that result from the chain intramolecular oxidation of the drug. The higher the HO<sup>•</sup> yield, the higher the antimalarial activity of the compound. The other, less significant factor is generation of other radicals (RO<sup>•</sup>, RO<sub>2</sub><sup>•</sup> LS<sup>•</sup>), which also kill the plasmodium, but do this less effectively. In terms of  $IC_{50}^{•}$ , these radicals are 1–2 orders of magnitude less effective than the hydroxyl radical. The peroxides generating a single radical are practically inactive against the plasmodium.

The kinetic analysis of radical reactions, which result from the transformations of the *hybrid peroxide antimalarial compounds* revealed the two ways of action, one being caused by the hydroxyl radicals alone and the other one that does not involve radical generation. This method was used for differentiation of the way of therapeutic action for a series of ten model compounds, which were synthesized starting from 10-dihydroartemisinine. The therapeutic action of several compounds evidenced the additional effect of the substituent, and for the others the analysis revealed the ability of the corresponding substituents to decrease the therapeutic effect, which is caused by the generation of hydroxyl radicals.

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