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Influence of stereoisomer of dispiro-1,2,4,5-tetraoxanes on their binding mode with heme and on antimalarial activity: molecular docking studies

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Abstract—Based on the fact that different isomers may exhibit substantial distinct activities, quantum chemical calculations and automated molecular docking simulations were carried out for 13 dispiro-1,2,4,5-tetraoxane compounds, which experimentally exist as a mixture of several isomers, to elucidate the most probable isomer(s) responsible for their antimalarial activity. The results indicate significant effects of stereoisomer on the binding mode and the activity. Moreover, the antimalarial potency of each compound can be described by the docking results. Compounds **1**, **2**, **4**, **5**, **7**, and **9** have the most probable isomers coordinate suitably with heme iron and hence they have high activities while the most probable isomer in compounds **3** and **8** could not bind appropriately to heme yielding only moderate activities. On the other hand, the steric hindrance in compounds **11**–**13** prevents an approach of heme iron to peroxide bonds resulting in a devoid of antimalarial activity. However, compounds **6** and **10** with isopropyl substituents exhibit a different docking character, which is possibly caused by a limitation in molecular flexibility of the available docking technique. Our results can be used as a guideline for stereochemical control in synthesis process to improve drug's potency.

1. Introduction

Malaria is the most important tropical disease, which causes an extremely serious health problem in many developing countries worldwide. Around 40% of the world's population in 100 countries are at risk and the disease is estimated to cause around 300–500 million illnesses and more than 1 million deaths each year.[1] One of the most crucial obstacles for eradicating malaria is a widespread resistance of malarial parasite to almost all chemotherapeutic agents. Therefore, it is very necessary to seek for new drugs that are effective against drug-resistant strains in order to combat and relieve this tremendous prevalence.

Artemisinin (Fig. 1A) and its derivatives are only a group of antimalarial compounds with no clinical report of resistance.[2] Unlike the already resisted antimalarial agents that contain nitrogen atom, these compounds have an endoperoxide moiety as critical pharmacophoric functional group, which may result in different mode of action and hence effective against drug-resistant strains. Although these compounds have been widely used in many countries, the current situation of malaria is still not significantly improved. The main reason is that artemisinin compounds are very expensive for most patients who are poor people. Therefore, new effective antimalarial drug with lower price is urgently needed. Currently, researchers are focusing on compounds

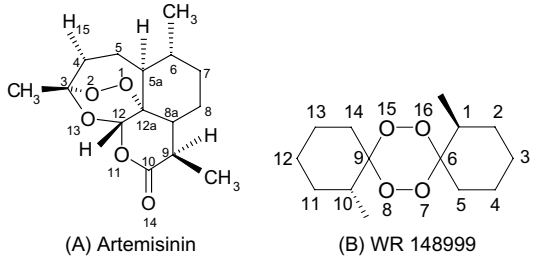

Figure 1. Structure of (A) artemisinin and (B) dispiro-1,2,4,5-tetraoxane WR 148999 with atom numbering.

Keywords: Molecular docking; Antimalarial activity; Dispirotetraoxane; Stereoisomer.
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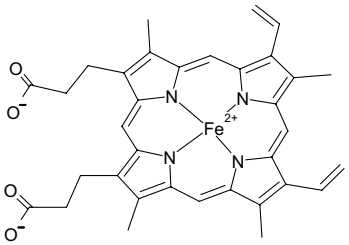


Figure 2. Structure of the heme molecule.

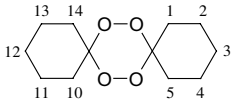
containing peroxide bond but having simpler structure than artemisinin. Dispiro-1,2,4,5-tetraoxanes, such as WR 148999[3] (Fig. 1B), are promising compounds in that they have simple structure, are easily prepared from relatively cheap raw materials,[4] and some of them possess good antimalarial activity.[5]

Since tetraoxanes contain peroxide groups, their mechanism of action is believed to be similar to that of artemisinin. The peroxide linkage is activated by ferrous ion, most probably a heme iron (Fig. 2), to produce oxygen free radicals that are rearranged to form carbon free radicals. Subsequently, these carbon reactive species alkylate specific malarial parasite proteins interrupting their normal functions and causing the parasites to die.[6,7]

In a synthesis process of dispiro-1,2,4,5-tetraoxane derivatives, it is usually not practical to apply stereochemical control so most products exist as a mixture of several isomers.[5] Since an isolation of each single isomer is very difficult, an antimalarial activity of a compound is generally measured from the mixture of many isomers although it is well known that different isomers may exhibit totally opposite results. Therefore, theoretical calculation is required for predicting a potency of each individual isomer. Based on the fact that drug should appropriately bind with a specific target, a receptor, in order to mediate its effects, molecular docking technique is used to elucidate proper drug–receptor interactions and configuration. This method can give a reasonable guideline, which isomer is the most active and thus whether it is worth to isolate that individual isomer. Moreover, the obtained docking results will enhance an understanding of drug–receptor interactions, which enables a modification of the drug's structure to achieve suitable interactions. Hence, this can bring about a development of new and more effective drugs. In this study, theoretical docking technique was applied to 13 dispiro-1,2,4,5-tetraoxane derivatives taken from the literature.[5] Their structures and antimalarial activities are given in Table 1.

The absolute configuration of each compound was not experimentally elucidated so all possible isomers should be considered. However, the disprio-1,2,4,5-tetraoxane, which contains three 6-membered ring systems, has very high flexibility, and it is not possible to explore all possible conformation of these three rings within a scope of this study. Therefore, the conformation of these three rings, for all compounds used in this study, is fixed to

Table 1. Structure and antimalarial activity of 13 dispiro-1,2,4,5-tetraoxanes



No	R	IC_{50} (nM)
1	2,11-Dimethyl	76
2	3,12-Dimethyl	23
3	3,12-Di-*tert*-butyl	200
4	2,3,11,12-Tetramethyl	12
5	2,4,11,13-Tetramethyl	30
6	1,10-Diisopropyl-4,13-dimethyl	47
7	1,2,10,11-Tetramethyl	50
8	1,3,10,12-Tetramethyl	110
9	1,4,10,13-Tetramethyl	15
10	4,13-Diisopropyl-1,10-dimethyl	140
11	1,3,3,10,12,12-Hexamethyl	>1000
12	3,3,12,12-Tetramethyl	>1000
13	2,2,4,4,11,11,13,13-Octamethyl	>1000

those of the X-ray structures of three dispiro-1,2,4,5-tetraoxane compounds,[4] that is, all three rings have chair conformations and the two cyclohexane rings are in *trans*-positions with regard to the tetraoxane ring thus yielding an inversion symmetry (see Fig. 3). As a consequence of this inversion symmetry, any two compounds with identical substituents but at opposite positions, for example, 1α versus 10β, 2α versus 11β, 3α versus 12β, 4α versus 13β, and 5α versus 14β, will be enantiomers of each other.

Experimentally, compounds **1**–**5** were formed without stereochemical control and were isolated as mixtures of at least two diastereomers. Compounds **1**, **2**, and **3** have two substituted positions, therefore, four isomers are feasible for each compound while compounds **4** and **5** have four functional groups giving a possibility of 16 isomers. Tetraoxane **6** was formed as a single stereoisomer but without centrosymmetry. Thus, only 12 isomers are possible. For compounds **7**–**11**, they exist as a single stereoisomer, presumably with centrosymmetric

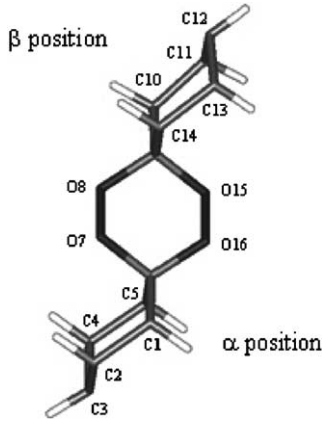


Figure 3. Stereochemistry of dispiro-1,2,4,5-tetraoxane compound showing substituted positions that have steric hindrance to both O–O bonds.

property and this limits the possibility to only four isomers for each compound. Compounds **12** and **13** have no isomer.

2. Results and discussion

For a comparison purpose, in each compound, energy was reported as a relative energy (ΔE) to its most stable isomer and results were reported in an ascending manner with respect to the energy. Our previous studies[8,9] showed that interactions between peroxide linkage in artemisinin compounds and heme iron play major role in the binding mode, therefore, distances between heme iron and four peroxide oxygens, O7, O8, O15, and O16 as well as a binding energy (B.E.) were monitored. A frequency of the most occurring configuration (denoted as 'Freq.') is also reported. All the data are given in Table 2.

Most compounds in this study have various possible isomers so their stereochemistries were examined. All 13 tetraoxane compounds have the same core structure in which experimentally all three rings are in the chair

Table 2. Relative energy and docking results between heme and dispiro-1,2,4,5-tetraoxanes

No	Configuration	ΔE (kcal/mol)	Freq. (%)	B.E. (kcal/mol)	O7–Fe (Å)	O8–Fe (Å)	O15–Fe (Å)	O16–Fe (Å)
1	2α11β	0.00	38	−31.27	2.56	2.21		
			30	−31.09			2.50	2.22
	2α11α	2.11	53	−30.54	2.70	2.17		
	2β11β	2.11	64	−29.69			2.45	2.39
	2β11α	4.21	29	−26.71			3.23	2.25
2	3β12α	0.00	73	−30.49			2.64	2.16
	3α12α	1.62	47	−29.64	2.66	2.23		
	3β12β	1.62	54	−28.87			2.49	2.37
	3α12β	3.24	69	−30.58	2.71	2.19		
3	3β12α	0.00	29	−29.36	4.52	4.04		
	3α12α	6.03	38	−33.94	2.63	2.19		
	3β12β	6.03	43	−33.27			2.52	2.30
	3α12β	12.06	34	−33.03	2.68	2.59		
4	2α3β11β12α	0.00	47	−32.94			2.61	2.18
	2α3α11β12α	1.65	47	−32.59	2.57	2.18		
	2α3β11β12β	1.65	30	−32.34			2.58	2.11
	2α3β11α12α	2.38	56	−32.97	2.58	2.13		
	2β3β11β12α	2.38	61	−32.97			2.70	2.18
	2α3β11α12β	2.67	22	−29.26	2.59	2.67		
	2β3α11β12α	2.67	28	−29.31			2.89	2.45
	2α3α11β12β	3.31	52	−32.67	2.57	2.30		
	2β3β11β12β	4.03	61	−32.41			2.47	2.10
	2α3α11α12α	4.03	63	−32.15	2.66	2.15		
	2β3α11β12β	4.32	62	−30.86			2.52	2.41
	2α3α11α12β	4.32	57	−31.36	2.66	2.37		
	2β3β11α12α	4.75	34	−28.89			3.17	2.24
	2β3β11α12β	5.04	44	−28.31			3.20	2.25
	2β3α11α12α	5.04	22	−27.96	3.24	2.23		
	2β3α11α12β	5.33	22	−27.38	3.10	2.42		
5	2α4α11β13β	0.00	41	−34.30			2.55	2.13
	2α4α11α13β	2.09	34	−33.21	2.67	2.15		
	2β4α11β13β	2.09	51	−33.81			2.42	2.32
	2α4α11β13α	2.16	33	−32.93	2.70	2.22		
	2α4β11β13β	2.16	31	−33.34			2.51	2.26
	2β4α11α13β	4.17	26	−29.03			3.06	2.22
	2α4β11α13β	4.24	31	−29.05	2.93	2.66		
	2β4α11β13α	4.24	30	−29.90			2.73	2.70
	2α4β11β13α	4.31	28	−29.36	2.68	2.70		
	2α4α11α13α	6.79	59	−32.57	2.68	2.13		
	2β4β11β13β	6.79	52	−33.13			2.42	2.45
	2β4α11α13α	8.87	27	−28.45	3.29	2.26		
	2β4β11α13β	8.87	40	−28.42			3.21	2.26
	2α4β11α13α	8.94	32	−28.50	2.74	2.69		
	2β4β11β13α	8.94	21	−28.58			2.80	2.74
	2β4β11α13α	13.56	23	−26.58	3.25	2.59		
6	1β4α10β13β	0.00	23	−31.80			2.52	2.76
	1β4α10α13α	1.43	18	−33.31	2.69	2.55		
	1β4β10α13β	1.43	26	−33.65			2.73	2.71
	1β4β10β13β	2.37	36	−31.54			2.46	2.91
	1β4α10β13α	2.51	33	−31.77			2.71	2.76

(*continued on next page*)

Table 2 (*continued*)

No	Configuration	ΔE (kcal/mol)	Freq. (%)	B.E. (kcal/mol)	O7–Fe (Å)	O8–Fe (Å)	O15–Fe (Å)	O16–Fe (Å)
	1α4α10α13β	2.76	29	−31.97	2.46	2.83		
	1β4β10β13α	4.88	16	−31.65	5.60	5.27		
	1α4β10α13β	4.99	48	−31.68	2.74	2.73		
	1α4α10α13α	5.13	27	−31.08	2.57	2.79		
	1α4β10β13β	6.07	22	−30.78	2.73	3.17		
	1α4α10β13α	6.29	14	−31.02	4.56	4.80		
	1α4β10α13α	7.37	20	−30.84	2.71	2.78		
7	1β2α10α11β	0.00	48	−34.85			2.41	2.28
	1α2α10β11β	2.05	20	−26.69			4.69	4.69
	1β2β10α11α	4.03	29	−28.31			3.19	2.42
	1α2β10β11α	4.69	21	−24.20	6.18	6.37	5.52	5.29
8	1β3β10α12α	0.00	30	−33.71			2.54	2.33
			26	−33.71	2.67	2.26		
	1α3β10β12α	2.55	25	−26.98			2.64	3.30
	1β3α10α12β	3.29	52	−34.17	2.66	2.26		
	1α3α10β12β	11.20	29	−27.53			2.67	3.03
9	1β4α10α13β	0.00	44	−35.42			2.51	2.22
	1α4α10β13β	2.47	17	−26.95	2.62	2.98		
	1β4β10α13α	4.43	28	−29.34	2.92	2.63		
	1α4β10β13α	7.19	26	−28.00	2.85	3.32		
10	1β4α10α13β	0.00	34	−37.95			2.67	2.21
	1α4α10β13β	2.59	13	−29.98	2.70	3.03		
	1β4β10α13α	4.34	21	−31.73	2.92	2.74		
	1α4β10β13α	6.26	20	−31.88			2.73	3.10
11	1β10α	0.00	25	−29.69			2.88	2.37
	1α10α	4.25	25	−27.53	3.22	2.44		
	1β10β	4.25	21	−27.95			3.32	2.48
	1α10β	8.52	15	−28.15			2.84	3.02
12	—	—	55	−28.31			2.83	2.36
13	—	—	32	−29.08			3.14	2.71

forms as shown in Figure 3. For clarity, only substituted positions producing steric hindrance to the O–O bonds are presented. Substituent pointing to a left-hand side (see Fig. 3) is in a β-position while that to a right-hand side is in an α-position. It is clearly seen that large substituent group at 1α- or 5α-positions and at 11α-, 12α-, or 13α-positions will hinder the binding of heme iron to O16 and O15 atoms, respectively. Thus, long O15–Fe and O16–Fe distances are expected for compound with large substituent(s) at these positions. Similarly, bulky functional group at 2β-, 3β-, or 4β-positions and at 10β- or 14β-positions limits the approach of Fe to O7 and O8 atoms, respectively, which will result in long O7–Fe and O8–Fe distances. The effect from steric group at 3β- and 12α-positions should be weaker because it is quite far from the peroxide bonds.

As stated above that the stereochemistry of each isomer has an effect on the binding, it is better to divide compounds into four groups according to their isomer characteristics and to discuss each group separately. The first group contains compounds that are a mixture of at least two isomers, that is, compounds **1**–**5**. Compound **6** in the second group is a single non-centrosymmetric stereoisomer. For the third group (compounds **7**–**11**), each compound exists as a single centrosymmetric isomer. Compounds in the final group (compounds **12** and **13**) do not have any isomer because both α- and β-positions are identically occupied.

2.1. Compounds as a mixture of at least two isomers (1–5)

Antimalarial activity of compounds **1**–**5** was evaluated from a mixture of at least two isomers.[5] Therefore, an existing probability of each isomer was determined based on an energetic point of view. Subsequently, a docked structure of each possible isomer was discussed.

Considering the relative energy of all four isomers of compound **1**, an isomer 2α11β is the most stable isomer. However, isomers 2α11α and 2β11β, which are enantiomers of each other, have ΔE of only 2.11 kcal/mol, therefore, it is possible that all these three isomers coexist in a solution. For docking results, methyl groups at both 2α- and 11β-positions in the isomer 2α11β do not cause steric hindrance to the two peroxide bonds so the heme iron can approach the compound at both O7–O8 and O15–O16 bonds as indicated by comparable occurring frequencies between the two docked structures (see Table 2). The most occurring docked structure, which has O8–Fe as the shortest distance, is displayed in Figure 4. It should be noted that this docked structure is postulated as a transition state geometry in the reaction mechanism. In the isomer 2α11α, heme iron attacks the compound favorably at O7–O8 bond due to no steric hindrance at this side. The O8–Fe distance is comparable to that of isomer 2α11β, 2.17 versus 2.21 Å. Although the isomer 2β11β has equal energy to the isomer 2α11α, it docks quite differently to heme. The O15 atom plays more contributions on the binding. The iron is

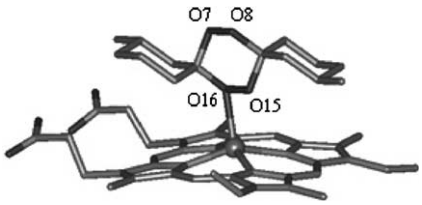


Figure 4. Docking structure between heme and 2α11β isomer of tetraoxane **1**.

positioned at around the middle of O15–O16 bond giving comparable O15–Fe and O16–Fe distances, 2.45 versus 2.39 Å. In the case of isomer 2β11α, it has steric hindrance from methyl groups at both 2β- and 11α-positions, however, its most frequently occurring configurations have O16–Fe as the shortest distance with value of 2.25 Å, which is comparable to other isomers. Considering its 3D structure (Fig. 3), the methyl substituent at 11α-position has profound steric effect on the O15 atom but not on the O16 atom. Therefore, iron can still attack the O16 atom though with less opportunity as indicated by the occurring frequency of only 29% and the binding energy of only −26.71 kcal/mol. In addition, this also resulted in a very long O15–Fe distance (3.23 Å) as expected.

Compound **2** has less energy differences among the four isomers than compound **1** so its antimalarial activity may be measured from a mixture of all four isomers. Isomers with methyl group at the 3β-position, that is, 3β12α and 3β12β, has steric hindrance on O7–O8 bond, therefore, heme iron binds to the compound at O15–O16 bond. Although the isomer 3β12α, the most stable isomer, has methyl group at 12α-position, it can still dock well to heme as evidenced by the O16–Fe distance of 2.16 Å and the occurring frequency of 73%. The weaker steric effect at 12α-position is probably a result of a longer distance between C12 atom and O15–O16 bond. The isomer 3β12β has a longer O16–Fe distance due to an increasing role of O15 atom in the binding. An isomer 3α12β, which has the highest energy ($\Delta E = 3.24$ kcal/mol), can coordinate to heme with the shortest O–Fe distance of 2.19 Å. The close contact of isomers 3β12α and 3α12β to heme may be a reason for their high antimalarial potencies (IC_{50} of 23 nM).

For compound **3**, an isomer 3β12α is the most stable isomer and is much more stable than the remaining three isomers with energy differences of 6.03 and 12.06 kcal/mol. However, the experimental data indicated the present of at least two diastereomers,[5] therefore, from the energetic point of view isomers 3α12α and 3β12β can exist while an isomer 3α12β with ΔE of 12.06 kcal/mol would be prohibited. The two *tert*-butyl substituents, which are more bulky than the methyl group, induce profound steric effects on the heme binding. For instant, long O–Fe distances (4.04 and 4.52 Å) were observed for the isomer 3β12α. As the heme iron could not access to peroxide bond in the isomer 3β12α, the most populated isomer in solution, this compound should devoid of antimalarial activity. However, the second most populated isomers (3α12α and 3β12β) can react with heme iron. This is probably a reason for low biological activity of this compound (IC_{50} of 200 nM). The isomer 3α12β cannot dock well with heme but this does not have any influence to the activity because this isomer does not exist in experimental solution.

Compound **4** with methyl groups at four positions have totally 16 isomers. The highest energy isomer, the isomer 2β3α11α12β, has ΔE of only 5.33 kcal/mol so the experimental solution can contain all isomers. However, a mixture of isomers 2α3β11β12α, 2α3α11β12α, and 2α3β11β12β is thermodynamically most conceivable. All these three isomers bind tightly with heme iron as certified by short O–Fe distances and low binding energies. Therefore, this compound is highly effective against malarial parasite (IC_{50} of 12 nM). It is interesting to note that the steric hindrance at both 3β- and 12α-positions have less effect on the binding since these three isomers can still dock well to heme. This is because the substituent at the two positions is quite far from the O–O bonds (Fig. 3). Although there are some isomers other than the above three isomers that do not suitably bind to heme, they have no substantial contribution to antimalarial activity due to their relatively small amounts in solution.

Unlike the previous compound, tetraoxane **5** with four methyl substituents has a large energy deviation among 16 isomers. The maximum ΔE is as high as 13.56 kcal/mol and only 5 out of 16 isomers have ΔE values of less than 3 kcal/mol. Thus, the reaction products from experiment should presumably consist of only these five isomers. The most stable isomer, 2α4α11β13β, has the most frequently occurring configuration with O16–Fe distance of only 2.13 Å. Interestingly, all these five isomers bind well to heme while most of the other 11 isomers do not. Compound **5** is slightly less active than compound **4** (IC_{50} of 30 nM vs 12 nM). The reason can perhaps be directed from the docking results. Among the five possible isomers, two of them, 2β4α11β13β and 2α4β11β13β, have slightly longer O16–Fe distances than those do in compound **4**.

2.2. Compound as a single non-centrosymmetric isomer (6)

According to the experimental data that compound **6** was isolated as single isomer without a centrosymmetry, only 14 isomers are possible. All available isomers have at least one substituent at the hindrance positions: 1α, 4β, 10β, and 13α. As the isopropyl group is quite bulky, it prevents the binding to heme iron and all 14 isomers have long O–Fe distances. Therefore, its high antimalarial activity (IC_{50} of 47 nM) could not be described from the docking results. The reason should be owing to a technical limitation of the software used in this study. A flexibility of receptor molecule is not allowed in the AutoDock 2.4,[10] therefore, a search for suitable binding structure is limited. More sophisticated method, such as molecular dynamics (MD) simulations, is required in this case. However, the computational cost of such method is extremely expensive compared to our docking

method so it is not applicable in this studies, which have a lot of structures.

2.3. Compounds as a single centrosymmetric isomer (7–11)

From experimental data, compounds **7**–**11** exist in the solution as a single isomer, which is presumably a centrosymmetry diastereoisomer,[5] but their absolute configurations were not established. Therefore, it is of interest to identify the structure of this single isomer by means of quantum chemical calculation. Isomer with the lowest energy, the most stable isomer, is theoretically the most probable candidate. The docking characteristic of this isomer was investigated in order to find relationship to its antimalarial activity.

Compound **7** has four substituents at positions 1, 2, 10, and 11 but only four isomers are possible due to the centrosymmetric property of positions 1 and 10, and of positions 2 and 11. Among these four possible isomers, an isomer 1β2α10α11β has the lowest energy, therefore, it is likely to be a configuration presented in the experimental solution. However, an isomer 1α2α10β11β is another possible configuration because it has ΔE value of only 2.05 kcal/mol, while the other two remaining isomers have higher ΔE and should exist in a very small fraction in the solution. The profound effect of substituents at positions 1α and 10β on docking structure is clearly seen in this compound. Both isomers 1α2α10β11β and 1α2β10β11α have unfavorable docked configurations as evidenced by long O–Fe distances and high binding energies. In the case of isomer 1α2β10β11α, it also has additional steric effect from substituents at the positions 2β and 11α resulting in the shortest O–Fe distance of 5.29 Å. The most stable isomer, 1β2α10α11β, has no steric so it can coordinate well with heme as indicated by O16–Fe distance of 2.28 Å. From the docking results and the fact that this compound has high antimalarial activity, we proposed that the experimental solution contains only the isomer 1β2α10α11β and the antimalarial activity should come solely from this isomer.

In tetraoxane **8**, a very high ΔE of isomer 1α3α10β12β (11.20 kcal/mol) prevents its existence. An isomer 1β3α10α12β with ΔE value of 3.29 kcal/mol is also unlikely to exist. Hence, only isomers 1α3β10β12α and 1β3β10α12α are candidate. The docking method could be used to justify which isomer should dominate. The isomer 1α3β10β12α does not dock well to receptor with the shortest O–Fe distance of 2.64 Å indicating no important interaction with heme and it should devoid of activity. However, the compound is moderately active; therefore, this isomer is neglected. The only one possibility is then the isomer 1β3β10α12α, which is also the most stable isomer. The docking calculations showed that its most occurring configuration (30%) has slightly long O16–Fe distance (2.33 Å). However, the second most frequently occurring configuration (26%) has shorter O8–Fe distance of 2.26 Å, which is in a range with other compounds. This is perhaps a reason for its less potency (IC_{50} of 110 nM). Considering the isomer 1β3α10α12β, which has no steric hindrance at both peroxide bonds but has high energy ($\Delta E = 3.29$ kcal/mol), it docks well to heme with O8–Fe distance of 2.26 Å and the occurring frequency of 52%. Thus, applying a diastereoisomeric control on this compound to give only the isomer 1β3α10α12β may enhance its antimalarial activity.

Compound **9** has an isomer 1β4α10α13β as the most stable structure. An isomer 1α4α10β13β is the second lowest energy with ΔE of 2.47 kcal/mol. These two isomers are energetically feasible while the other twos have much higher energies. Therefore, the experimental structure of this compound should be either 1β4α10α13β or 1α4α10β13β and docking results of only these two isomers are discussed. As in compounds **6**–**8**, substituents at positions 1α and 10β substantially block the approach of heme to both peroxide bonds resulting in long O–Fe distances. The isomer 1α4α10β13β has O7–Fe distance of 2.62 Å as the shortest distance, which does not imply any crucial interaction. However, based on the fact that compound **9** has high biological activity, this isomer should not exist and it is left out. The isomer 1β4α10α13β coordinates with heme iron using O16 atom. The O16–Fe distance of 2.22 Å and the binding energy of −35.42 kcal/mol indicate a good binding and hence high antimalarial activity (IC_{50} of 15 nM). Therefore, this isomer is likely corresponding to the experimental structure.

Similarly to compound **9**, the lowest energy structure of tetraoxane **10** is an isomer 1β4α10α13β. Also, their patterns of ΔE for each isomer are much alike. This may be because their structures are very similar. Only the most stable isomer contains no steric at the peroxide bonds. Therefore, its shortest O–Fe distance is 2.21 Å whereas it is around 2.7 Å for the other three isomers. From these results, the isomer 1β4α10α13β should be an isomer appeared in the solution. Although this isomer can coordinate to heme quite well, its antimalarial activity is not as active as other compounds that have the same docking manner. The reason is not clearly known but, however, it is likely that the two isopropyl substituents play a responsible role, as in the case of compound **6**, and the more sophisticated and expensive method such as MD simulation is required.

Although compound **11** has six methyl groups, only four isomers are possible because four of the methyl groups at both α- and β-positions of C3 and C12 atoms leads to no extra isomer. The energy of all four possible isomers indicates that an isomer 1β10α is the most stable and should be presented in the solution while the other three isomers with much higher energies are barely exist in experiment. Due to steric hindrance from methyl groups at positions 3β and 12α, all isomers have long O–Fe distances. Even the isomer 1β10α, which has no steric at both 1- and 10-positions, the shortest O–Fe distance is still quite long (2.37 Å). The steric hindrance is maximum in the case of an isomer 1α10β with the shortest O–Fe distance of 2.84 Å. Therefore, this compound is devoid of antimalarial activity regardless of its absolute configuration (isomer).

2.4. Compounds with no isomer (12 and 13)

For compounds **12** and **13**, no isomer is available because they have identical substituents at both α- and β-positions. Therefore, only one structure was investigated for each compound. Tetraoxane **12** has four methyl groups at positions 3 and 12. The O16–Fe distance of 2.36 Å and the binding energy of −28.31 kcal/mol indicate no significant interaction between tetraoxane **12** and heme. This is possibly a reason for its inactive potency. Comparing this compound to tetraoxane **11**, it docks with heme very similar to the isomer 1β10α of compound **11**. This confirms that the methyl groups at 1β- and 10α-positions do not play any role in the docking. The last compound, **13**, is the most bulky compound because of eight methyl substituent groups at positions 2, 4, 11, and 13 around the molecule. Therefore, heme cannot reach O–O bonds as demonstrated from the shortest O–Fe distance of 2.71 Å. The reaction responsible for antimalarial effect is not able to take place and hence the compound is relatively inactive.

3. Conclusion

Based on the fact that drug must bind with its receptor to mediate its biological effect, the docking calculations between 13 dispiro-1,2,4,5-tetraoxane compounds and heme were performed. All possible isomers of each compound were additionally investigated by means of quantum chemical method. As in our previous studies,[8,9] the interactions between heme iron and peroxide bond in tetraoxanes play a major role for antimalarial activity. The docking results indicate significant effects of steric hindrance on the binding and hence the biological activity. The relationship between steric hindrance and antimalarial activity was found. Compound in which its most stable isomer docks well with heme iron has high activity while compound with substituent(s) that hinders the approach of heme iron to O–O bonds is less effective and compound having substituent(s) with full blockage is devoid of potency. The functional groups at positions 1α and 10β that are juxtaposed to the peroxide bonds have profound effect on the docking configuration than those at the other positions do.

For compounds **1**–**5** that exist as a mixture of at least two isomers, possible isomers were determined from the energetic point of view and then only these isomers should be responsible for the antimalarial activity. If all of these structures bind well with heme, they will reinforce the high activity as in compound **4**. But if some isomers do not suitably interact with the receptor, the compound has less potency, for example, compound **3**. According to our docking results, a method to improve biological activity is suggested. By applying a stereochemical control in synthesis reaction to give only isomer(s) that docks properly to heme, the antimalarial activity could be enhanced.

All of isomers in tetraoxane **6** have at least one substituent hindering the peroxide bonds. Moreover, the isopropyl groups at positions 1α and 10β cause additional obstruction. Therefore, all 14 possible isomers have long O–Fe distances. Since this compound has quite high activity, the current docking method seems not possible to fully describe the activity. This maybe due to the limitation of AutoDock 2.4 that does not allow full flexibility of both molecules. Although MD simulation is more appropriate for this system, its computational cost is too expensive and thus makes it not feasible at the present time.

In the case of compounds **7**–**11**, which experimentally have only one isomer with centrosymmetry property, the most probable isomer of each compound was suggested based on theoretical calculations. The docking feature of this isomer has a relation with its antimalarial activity. The only exception is compound **10** that exhibits very similar manner to compound **6**. It is interesting to note that both compounds have the isopropyl substituents, which cause additional flexibility problem to the docked configuration than methyl group does. The docked structures of compounds **12** and **13** clearly demonstrate the relation to their biological activities. Both compounds could not dock well to heme iron and hence devoid of potency.

In summary, our studies clearly reveal the influences of stereoisomer of dispiro-1,2,4,5-tetraoxanes on their binding mode with heme and on the antimalarial activity. The data can be used to assist further improvement and development of new effective drugs, for example, by applying a stereochemical control in synthesis process.

4. Experimental

4.1. Biological data

The antimalarial activity of 13 dispiro-1,2,4,5-tetraoxane derivatives with alkyl substituents, which were measured in vitro as the IC_{50} values, the inhibitory concentration of a compound required for 50% inhibition of the parasitemia, against the Sierra Leone (D-6) clone of *Plasmodium falciparum*, were taken from the literature.[5]

4.2. Computational methods

All tetraoxane compounds were built using the X-ray structure of 1,2-dimethyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane (Fig. 1B) as a template. For each compound, all available isomers corresponding to experimental data were considered. Subsequently, geometries of all compounds were fully optimized at the ab initio Hartree Fock level with 3-21G basis set using the Gaussian 98 program.[11]

For docking calculations, the AutoDock 2.4, an automated docking program, was used. The program employs a simulated annealing Monte Carlo simulation in combination with a rapid grid-based energy evaluation method to search for the global minimum energy

docked configuration. The most appropriate parameter settings and procedures for docking determined from our previous studies of artemisinin compounds and heme[8,9] were employed as followings. The heme structure was modified from the X-ray structure of chlorohemin. A grid box of a dimension $25\times25\times25$ Å^3 with a spacing of 0.5 Å was used. The combined AMBER/MMFF parameters[12,13] were chosen for the Lennard-Jones 12,6 potentials and the Coulomb potentials instead of the AMBER force field alone that lacks specific parameter for iron. In one docking calculation, simulations start with an annealing temperature (RT) of 100 kcal/mol. When a ligand makes 30,000 accepted or 30,000 rejected moves, the temperature is reduced by a factor of 0.90, which is called 1 cycle. This process continues for 100 cycles. Finally, a structure corresponding to the most suitable docking configuration is obtained.

Actually, since the ligand can adopt many possible docked configurations to receptor, running only one calculation may not guarantee a good result. Therefore, 100 docking calculations were performed for every compound and a starting configuration was assigned in a random manner for each docking calculation to avoid any bias as well as to establish statistically reasonable results. All 100 docked configurations were clustered into groups. Configurations with root-mean-square-deviation (rmsd) values of less than 1 Å were grouped together. The lowest energy configuration was selected as a representative for each group. A group with the highest number of members, referred to as 'the most occurring configuration', is considered as it most probably represents the structure in a real system.

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