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Synthesis and Antimalarial Activity of Novel Bicyclic and Tricyclic Aza-peroxides

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Abstract For the first time, novel bicyclic **5a-h** as well as tricyclic **9a-h** aza-peroxides were synthesized using $^1O_2$-mediated photo-oxygenation methodology as key step in 52-71% yields in which one of the oxygen atom of 1, 2, 4-trioxane ring has been replaced by nitrogen atom. The methodology is simple and is an efficient way to access 1,2-dioxa-4-aza six membered ring compounds. All these compounds were assessed for their *in vitro* antimalarial activity against *Plasmodium falciparum.* Compound **9a**, **9c** and **9f**, the most active compound of the series, showed IC_{50} values of 9.43, 8.83 and 5.63 ng/ml, respectively which was found to be comparable to that of antimalarial drug Chloroquine (IC_{50} value 5.2 ng/ml). Compound **9f**, the most active compound found in *in vitro* studies, provided 40% protection to the infected mice at the dose of 96 mg/kg × 4 days when screened for its antimalarial activity *in vivo* against multidrug-resistant *Plasmodium yoelii nigeriensis* in Swiss mice by oral route. In this assay, β-arteether and Chloroquine, showed 100% suppression of parasitaemia on day 4 and provided 100% and 80% protection, respectively, to the infected mice.

Keywords: Malaria / Mitsunobu reaction/ Aza-peroxides / *in vitro* / antimalarial / Chloroquine

Introduction

Malaria continues to affect around 30% of world population and causes approximately 300−500 million clinical cases per year and nearly 584,000 death cases in 2013, predominantly among children under 5 years of age and pregnant women in sub-Saharan African countries.[1] Due to the increase in the movements of human population into malarial regions, variation in climate changes, and above all, proliferation of multidrug resistant Plasmodium falciparum (Pf) parasites; there is an increase in the growth of the malarial burden experienced in recent years.[2]

Artemisinin and its semi-synthetic derivatives i.e. artemether, arteether and artesunic acid (Figure 1) are currently the drugs of choice for the treatment of malaria caused by multidrug-resistant *Plasmodium falciparum*.[3] The peroxide group present in the form of 1,2,4-trioxane moiety of artemisinin is essential for the activity of this class of compounds. A wide range of artemisinin analogues had been prepared by several groups,[3] including our group,[3m-r] which showed very high order of antimalarial activity *in vivo* against multidrug-resistant malaria.[3] Unfortunately, artemisinin is associated with severe drawbacks such as limited supply, high cost, recrudescence, and recently indentified the neurotoxicity,[4] as well as nephrotoxicity, and the reported cases of clinical drug resistance[2] to currently used artemisinin derivatives strongly encourage the finding, of more viable alternatives to the existing chemotherapeutic treatments.

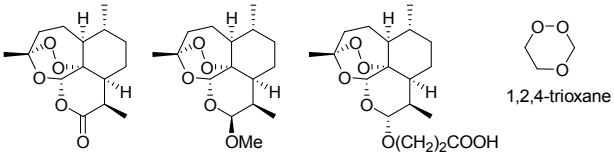

Figure 1. Artemisinin, its derivatives and its active pharmacophore.

In this regard, several structurally simple peroxides with promising *in vivo* antimalarial activity such as synthetic 1,2,4-trioxanes, 1,2,4,5-tetraoxanes, 1,2,4-trioxepanes, 1,2-dioxanes etc. have also been reported in the literature.[5-9] Many of these peroxides have shown high order of antimalarial activity more than clinically used β-arteether against multidrug-resistant malaria.[5-9] Parallelly, in recent years, several amino functionalized 1, 2, 4-trioxanes, reported by Singh et. al., have shown high order of *in vivo* antimalarial activity against multi-drug resistant *P. yoelii* in Swiss mice via oral as well as i.m. routes.[10]

Several attempts have been made to modify the pharmacophoric 1, 2, 4-trioxane moiety of structurally simple 1, 2, 4-trioxanes and assess

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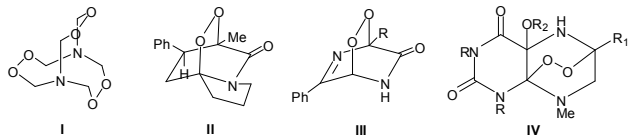
these new compounds for their antimalarial efficacy. This led to the synthesis and antimalarial testing of various classes of organic peroxides other than 1,2,4-trioxanes, such as endoperoxides, alkyl hydroperoxides, peroxyamines, peroxyketals, *β*-peroxy-lactones, etc.[11] With the peroxide bond being an indispensable requirement for the antimalarial activity, replacement of O4 of 1,2,4-trioxane by another heteroatom, mainly by nitrogen, was of obvious interest. Particularly relevant to this was, photooxygenation of allyl amine by Adams.[12a] Various other structural scaffolds eg. **I-IV**, with nitrogen as a part of the ring containing the peroxide bond have been reported.[12b-i] Most of these are endoperoxides formed by Diels-Alder reaction of a suitable diene with singlet oxygen.



Some other peroxides containing nitrogen atom in the whole molecule have also been reported, but the antimalarial activity of these aza-peroxides were not studied.[12j-k] Hence, there is still room for the detailed study of aza-peroxides containing nitrogen in the peroxide vicinity and could be prominent area of investigation.

As a part of an endeavor to develop novel antimalarial agents which can serve as structurally simple substitutes for artemisinin derivatives, we were interested in replacing the oxygen atom at 4th position of the 1,2,4-trioxane ring with nitrogen atom in order to study structure-activity relationship of newly synthesized skeleton (Figure 2). The idea was inspired from the fact that Aza-artemisinin, C-11 oxygen replaced with nitrogen atom, displayed 4 times more *in vivo* antimalarial activity against multidrug-resistant *P. yoelii* (Figure 2).[13] Herein, we report the synthesis and antimalarial activity of a new series of bicyclic and tricyclic aza-peroxides.

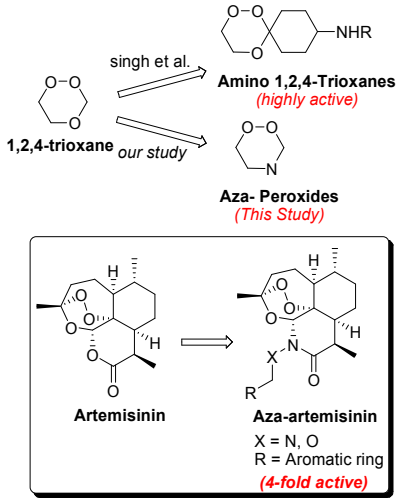
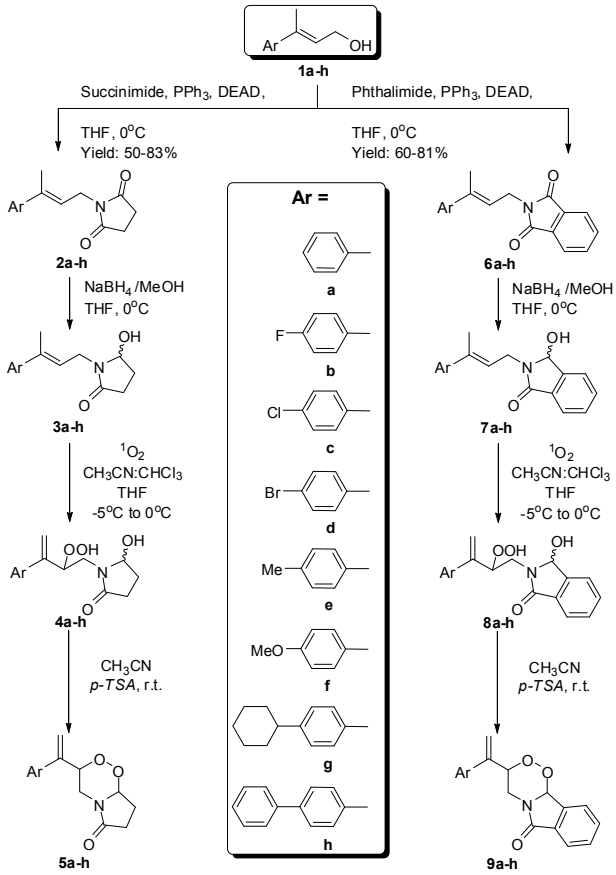


Figure 2. Comparative study.

Result and Discussion

In order to test our idea, we started our investigation with the synthesis of structurally simple bicyclic **5a-h** and tricyclic **9a-h**, respectively as shown below in the scheme 1.

The starting material allylic alcohols **1a-h,** [14] were subjected under Mitsunobu conditions with succinimide to furnished *N*-substituted succinimides **2a-h** in 60-74% yields. Similarly, the corresponding *N*-substituted phthalimides **6a-h** in 66-86% yields was also prepared with phthalimide under the same Mitsunobu conditions (Scheme 1). $NaBH_4$ reduction of imides **2a-h** and **6a-h** in a 1:1 mixture of THF and MeOH at 0^{o}C furnished hydroxy-functionalized lactams **3a-h** and **7a-h**, respectively in 58-74% yields (Scheme 1). Using the literature procedure, singlet oxygen-mediated Photooxygenation of **3a-h** and **7a-h** in a 2:1:1 mixture of CH_3CN, THF and $CHCl_3$ at -5 to 0^{o}C furnished the corresponding hydroperoxides **4a-h** and **8a-h** which, without purification, were subjected to acid catalyzed intramolecular cyclization to yield the corresponding dioxa-aza-indenones **5a-h** and dioxa-aza-fluorenones **9a-h**, respectively in 52-71% yields (Scheme 1, Table 1).[15]



Scheme 1. Synthesis of compounds **5a-h** and **9a-h.**

The acid-catalyzed intramolecular cyclization, to synthesize the corresponding dioxa-aza-indenones **5a-h** and dioxa-aza-fluorenones **9a-h**, was found to be highly diastereoselective as all these compounds were obtained as single isomers instead of pairs of diastereomers.

Table 1. Melting points and yields of dioxa-aza-indenones **5a-h** (bicyclic) and dioxa-aza-fluorenones **9a-h** (tricyclic)

Entry	Ar =	m.p. (^{o}C)	Yield (%)[a]
5a	C_6H_5-	oil	67
5b	4-fluoro-C_6H_4-	65-67	59

5c	4-chloro-C_6H_4-	69-71	55
5d	4-bromo-C_6H_4-	oil	52
5e	4-methyl-C_6H_4-	93-95	69
5f	4-methoxy-C_6H_4-	78-80	63
5g	4-cyclohexyl-C_6H_4-	81-83	59
5h	4-phenyl-C_6H_4-	136-138	71
9a	C_6H_5-	85-87	60
9b	4-fluoro-C_6H_4-	80-82	59
9c	4-chloro-C_6H_4-	121-123	54
9d	4-bromo-C_6H_4-	132-134	61
9e	4-methyl-C6H4-	oil	70
9f	4-methoxy-C6H4-	115-117	63
9g	4-cyclohexyl-C6H4-	135-137	57
9h	4-phenyl-C6H4-	148-150	61

a: yields based on hydroxy-functionalized lactams **3a-h** and **7a-h** as starting materials.

To find out the reason for this selectivity, we calculated the energies of two diastereomers of **5a** and **9a** using MOE (Molecular Operating Environment). The result of this study is shown in figure 3.

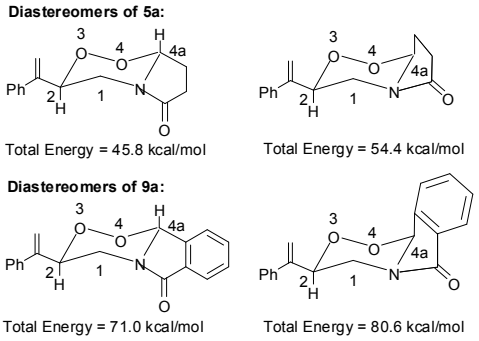


Figure 3. Energies of diastereomers of **5a** and **9a** as determined by MOE.

As can be seen from the figure 3; the formation of single diastereomer of **5a** and **9a** with aryl-vinyl group in equatorial position and 4aH in axial position is favored over the other diastereomers thereby confirms its selectivity. This was further confirmed by NOESY spectrum of **9g** which shows correlation between axial proton at C1 and proton at C4a (Figure 4).

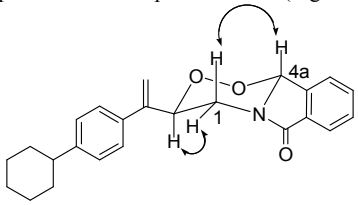


Figure 4. Representation of NOESY correlations in **9g.**

All of these aza-peroxides **5a-h** and **9a-h** were initially screened for their in vitro antimalarial activity against chloroquine-sensitive *P. falciparum* (3D7 strain).[16] Chloroquine and *β*-artemether served as positive controls for *in vitro* screening. The results are shown in Table 2. Out of these 16 compounds, among **5a-h** series; compounds **5g** and **5h**, and among **9a-h** series; the three most active compounds i.e. **9a**, **9c**, **9f** and the next active compound **9g** were selected for *in vivo* testing and screened against multidrug-resistant *P. yoelii nigeriensis* in Swiss mice by oral routes at the dose of 96 mg/kg × 4 days using Peter's procedure.[17] *β*-arteether served as a positive control for in vivo screening. The results are shown in Table 3.

Table 2. *In vitro* antimalarial activity of compounds **5a-h** and **9a-h** against *Plasmodium falciparum* 3D7 strain.

compd. no.	Log P[a]	IC_{50} (ng/mL)
5a	1.92	145.97
5b	2.07	162.59
5c	2.47	38.05
5d	2.74	158.61
5e	2.40	68.11
5f	1.79	245.89
5g	3.9	32.46
5h	3.59	15.27
9a	3.54	9.43
9b	3.70	39.58
9c	4.10	8.83
9d	4.37	60.11
9e	4.03	86.59
9f	3.41	5.63
9g	5.33	17.46
9h	5.22	45.36
Artemether	3.51	0.4
Chloroquine	3.73	5.2

[a]log P values are calculated using Chemdraw ultra 11.0.
[b]IC_{50}: concentration corresponding to 50% inhibition of chloroquine-sensitive strain 3D7 of *Plasmodium falciparum.*

As seen from table 2, despite having an entirely different pharmacophoric scaffold, these new organic peroxides showed significant antimalarial activity against *P. falciparum* in vitro. These new peroxides showed IC_{50} values within the range of 5.63 ng/ml - 245.89 ng/ml. The Dioxa-aza-fluorenones derived aza-peroxides **9a-g** displayed noticeably superior antimalarial activities (IC_{50} values within the range of 5.63 ng/ml - 60.11 ng/ml) as compared to the corresponding Dioxa-aza-indenones derived compounds **5a-g** (IC_{50} values within the range of 32.46 ng/ml - 245.89 ng/). The only exception to this trend, the compound **5h**, showed IC_{50} value of 15.27 ng/ml, which is lower than that of the corresponding phthalimide derived analogue **9h** having IC_{50} value of 45.36 ng/ml. Further, increasing lipophilicity (higher log P values), seems to be beneficial for enhancing the antimalarial activity of compounds of this series. Thus, compounds **5a-5f**, having log P values in the range of 1.79 - 2.74, showed poor antimalarial activities (IC_{50} = 38.05 ng/ml - 245.89 ng/ml). With increase in the lipophilicity and hence in log P values in the range of 3.41 - 5.33, compounds **5g**, **5h** and **9a-h**, showed parallel rise in antimalarial activities and displayed 50% inhibition of parasites at considerably lower concentrations (IC_{50} = 5.63ng/ml - 60.11 ng/ml).

Dioxa-aza-fluorenones derived aza-peroxides **9a**, **9c** and **9f**, demonstrated impressive antimalarial activities with IC_{50} values of 9.43 ng/ml, 8.83 ng/ml and 5.63 ng/ml, respectively. The most active azaperoxide **9f**, showed the highest *in vitro* antimalarial activity and

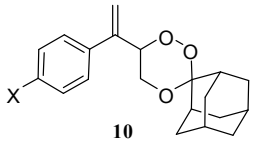
provided 50% inhibition of parasites at the concentration value of 5.63 ng/ml. Clinically useful drugs, artemether and chloroquine, showed 50% inhibition of the parasites at 0.4 and 5.2 ng/ml, respectively. Thus, compound **9f** is as active as chloroquine in the *in vitro* assay against *Plasmodium falciparum* 3D7 strain.

Table 3. Blood schizontocidal activity of **5g**, **5h**, **9a**, **9c, 9f** and **9g** against multidrug-resistant (MDR) strain *P. yoelii nigeriensis* in Swiss mice via oral route[17]

Compd.	Dose (mg / kg × 4 days)[a]	% suppression of Parasitaemia on day 4[b,c]	Cured / Treated[d]
5g	96	65	0/5
5h	96	57	0/5
9a	96	84	0/5
9c	96	89	0/5
9f	96	100	2/5
9g	96	100	1/5
***β*-arteether**	96	100	5/5
Chloroquine	96	100	4/5

[a]The drug dilutions of compounds were prepared in ground oil and administered to a group of mice at each dose, from day 0-3, once daily. [b]Parasitaemia levels were recorded from thin blood smears on day 4 and subsequently twice a week till day 28. Percent suppression= $[(C- T) / C] \times 100$; where C= parasitaemia in control group, and T= parasitaemia in treated group. [c]100% suppression of parasitaemia means no parasites were detected in 50 oil immersion fields during microscopic observation.[18] [d]"5/5" means none of the treated mice developed patent infection during the 28 days observation period and hence were recorded as cured. Similarly "0/5" means none out of 5 mice were found to be cured.

Compounds **5g**, **5h**, **9a**, **9c**, **9f** and **9g** were further tested *in vivo* against multidrug-resistant *Plasmodium yoelii nigeriensis* in Swiss mice by oral routes at the dose of 96 mg/kg × 4 days. As can be seen from table 3, compounds **5g**, **5h**, **9a** and **9c**, provided 65%, 57%, 84% and 89% suppression of parasitaemia, respectively, on day 4 at the dose of 96 mg/kg × 4 days by oral route. At the same dose, compounds **9f** and **9g** showed 100% suppression of parasitaemia on day 4. Further, compound **9g**, provided 20% protection to the infected mice, while compounds **9f**, provided 40% protection to the infected mice at 96 mg/kg × 4 days dose at the end of 28 day observation period. Clinically useful drugs, *β*-arteether and Chloroquine, showed 100% suppression of parasitaemia on day 4 and provided 100% and 80% protection, respectively, to the infected mice in this model. Thus, dioxa-aza-fluorenones derived compounds **9f** and **9g** showed moderate *in vivo* antimalarial activities against multidrug resistant *Plasmodium yoelii nigeriensis* in Swiss mice by oral route.



10

1,2,4-trioxanes (prepared by Singh et. al)

X = H, Me, OMe, F, Cl

Finally, it is worth to compare the antimalarial activity of these aza-peroxides with 1, 2, 4-trioxanes prepared by Singh et. al. While these aza peroxides **5a-h** and **9a-h** showed significant *in vitro* antimalarial activity; all the 1, 2, 4-trioxanes of prototype **10** prepared by Singh et. al were found to be *in vivo* active. This indicates that any change in the 1, 2, 4-trioxanes ring system, the basic pharmacophore responsible for antimalarial activity, would leads to decrease in the antimalarial activity of the compound which, thereby, underlines the importance of oxygen atom in 1, 2, 4-trioxane ring.

Conclusion

In conclusion, we report the synthesis of novel bicyclic aza-peroxides **5a-h** and tricyclic aza-peroxides **9a-h**, the two series of novel nitrogen analogues of 1, 2, 4-trioxanes in 52-71% yields. All these compounds were assessed for their *in vitro* antimalarial activity against *Plasmodium falciparum* 3D7 strain. Dioxa-aza-fluorenones derived compounds **9a**, **9c** and **9f** showed IC_{50} values of 9.43, 8.83 and 5.63 ng/ml, respectively which was found to be comparable to that of antimalarial drug Chloroquine (IC_{50} value 5.2 ng/ml). *In vivo* antimalarial activity of compound **9f**, the most active compound of the series, provided 40% protection to the infected mice at 96 mg/kg × 4 days dose at the end of 28 day observation period. To the best of our knowledge, this is a first attempt for the synthesis of dioxa-aza-indenones (bicyclic) **5a-h** and dioxa-aza-fluorenones (tricyclic) **9a-h** based aza-peroxides which replaces one oxygen atom of 1,2,4-trioxane moiety by incorporating nitrogen atom within active pharmacophore.

Experimental

General Methods

All glass apparatus were oven dried prior to use. Melting points were taken in open capillaries on complab melting point apparatus and are presented uncorrected. Infrared spectra were recorded on a Perkin-Elmer FT-IR RXI spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using $CDCl_3$ as solvent either using Bruker Supercon Magnet DPX-200 or DRX-300 spectrometers (operating at 200 and 300 MHz respectively for ^{1}H; 50 and 75 MHz respectively for ^{13}C) or using JEOL ECS-400 spectrometer (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C). Tetramethylsilane (δ 0.00 ppm) served as an internal standard in ^{1}H NMR and CDCl3 (δ 77.0 ppm) in ^{13}C NMR. Chemical shifts are reported in parts per million. Splitting patterns are described as singlet (s), doublet (d), triplet (t) and multiplet (m). Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained on JEOL SX-102/DA-6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Glycerol or *m*-nitrobenzyl alcohol was used as matrix. Electrospray mass spectrometry (ES-MS) were recorded on a MICROMASS QUATTRO II triple quadruple mass spectrometer. High Resolution Electron Impact Mass Spectra (HR-EIMS) were obtained on JEOL MS route 600H instrument as well as Xevo G2-S Q-Tof (Waters, USA). Elemental analyses were performed on Vario EL-III C H N S analyzer (Germany) and values were within ±0.4 % of the calculated values, and therefore these compounds meet the criteria of ≥95% purity. Column chromatography was performed over Merck silica gel (particle size: 60-120 Mesh and 100-200 Mesh) procured from Qualigens^{TM} (India), flash silica gel (particle size: 230-400 Mesh). All chemicals and reagents were obtained from Sigma Aldrich (USA), Merck (India) or Spectrochem (India) and were used without further purification.

General procedure for preparation of *N*-substituted imides 2a-h and 6a-h: Preparation of 2a as a representative:

To a stirred mixture of allylic alcohol **1a** (2 g, 13.5 mmol), succinimide (2.67 g, 26.99 mmol) and triphenylphosphine (8.85 g, 33.74 mmol) in dry THF (60 mL) at 0 ºC and under inert atmosphere, was added diethylazodicarboxylate (DEAD) (4.3 mL,

26.99 mmol) dissolved in dry THF (5 mL). The contents were stirred at 0 ºC for 30 min. THF was evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel (100-200 mesh) using 1.5% ethyl acetate: DCM as eluant to furnish 2.2 g (71% yield) of **2a**.

Compound 2a: (1-(3-Phenyl-but-2-enyl)-pyrrolidine-2, 5-Dione)
Yield: 71%; Solid, mp 129-131 ºC;
FT-IR (KBr, cm^{-1}) 1724.7, 1525.3, 1369.5, 1209.5, 766.1;
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12 (s, 3H), 2.64 (s, 4H), 4.23 (d, 2H, *J* = 6.8 Hz), 5.63-5.67 (m, 1H), 7.17-7.30 (m, 5H, Ar);
^{13}C NMR (100 MHz, $CDCl_3$) δ 16.23 (CH_3), 28.35 (2 × CH_2), 37.27 (CH_2), 120.43 (CH), 125.93 (2 × CH), 127.48 (CH), 128.31 (2 × CH), 139.84 (C), 142.70 (C), 176.96 (2 × C);
ESI-MS (m/z) 230 $[M+H]^+$.
Anal. calcd for $C_{14}H_{15}NO_2$: C, 73.34%, H, 6.59%, N, 6.11%; found: C, 73.51%, H, 6.61%, N, 6.19%.

N-substituted imides **2b-h** were synthesized from allylic alcohols **1b-h**, respectively by using the same procedure. Likewise, imides **6a-h** were synthesized from allylic alcohols **1a-h** by replacing succinimide with phthalimide.

General procedure for preparation of amides 3a-h and 7a-h: Preparation of 3a as a representative:

To the solution of *N*-substituted imide **2a** (2 g, 8.73 mmol) in MeOH: THF (1:1, 40 mL) at 0 ºC, was added sodiumborohydride (1.3 g, 34.93 mmol) in 5 fractions over a period of 2 h with continuous stirring. Reaction mixture was stirred at 0 ºC for 1 h. MeOH: THF was evaporated under reduced pressure to half of the original volume. Water (40 mL) was added and extracted with $CHCl_3$ (2 × 100 mL). Organic layer was dried over anhyd sodium sulphate and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel (100-200 mesh) using 1% Methanol: DCM as eluant to furnish 1.4 g (67% yield) of **3a**.

Compound 3a: (5-Hydroxy-1-(3-phenyl-but-2-enyl)-pyrrolidin-2-one)
Yield: 67%; Viscous oil; FT-IR (neat, cm^{-1}) 3776.4, 1687.5, 1660.4, 1209.7, 768.3;
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63-1.85 (m, 1H), 2.06 (s, 3H), 2.23-2.27 (m, 2H), 2.46-2.55(m, 1H), 2.98(s,1OH), 3.91-3.95 (m, 1H), 4.24 (dd, 1H, *J* = 6 and 15.2 Hz), 5.16 (s, 1H), 5.64-5.67 (m, 1H), 7.17-7.30 (m, 5H, Ar)
^{13}C NMR (100 MHz, $CDCl_3$) δ 16.10 (CH_3), 28.33 (CH_2), 28.95 (CH_2), 38.25 (CH_2), 83.06 (CH), 121.94 (CH), 125.83 (2 × CH), 127.47 (CH), 128.38 (2 × CH), 139.42 (C), 142.74 (C), 174.50 (C);
ESI-MS (m/z) 232 $[M+H]^+$.
Anal. calcd for $C_{14}H_{17}NO_2$: C, 72.70%, H, 7.41%, N, 6.06%; found: C, 72.85%, H, 7.56%, N, 6.25%.

Amides **3b-h** and **7a-h** were prepared from *N*-substituted imides **2b-h** and **6a-h**, respectively by using the same procedure.

General procedure for preparation of aza-peroxides 5a-h and 9a-h: Preparation of 5a as a representative:

A solution of amide **3a** (1 g, 4.33 mmol) and methylene blue (5 mg), in MeCN:THF:$CHCl_3$ (2:1:1, 60 mL) was irradiated with 500 W tungsten-halogen lamp at -10 to 0 ºC, while a slow stream of O_2 was bubbled into the reaction mixture for 6 h. The obtained crude reaction mixture of hydroxy hydroperoxide **4a** was concentrated under reduced pressure at r.t, dissolved in acetonitrile (100 mL) and *p*TSA (0.1 g) was added to it. The reaction mixture was stirred at 5 ºC for 2 h. The reaction mixture was concentrated to half of its original volume under reduced pressure at rt, diluted with water (100 mL) and extracted with ethyl acetate (2 × 75 mL). Combined organic layer was dried over anhyd sodium sulphate and concentrated under reduced pressure at rt. The crude product was purified by column chromatography over silica gel (60-120 mesh) using 10% ethyl acetate: hexane as eluant furnish pure product **5a** (0.69 g, 65% yield).

Compound **5a**: (5-(1-Phenyl-vinyl)-tetrahydro-6,7-dioxa-3a-aza-inden-3-one) Viscous oil;
FT-IR (neat, cm^{-1}) 1705.5, 1485.5, 1445.1, 1042.4, 773.9;
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74-1.84 (m, 1H), 2.24-2.49 (m, 3H), 3.13 (dd, 1H, *J* = 13.4 and 10.7 Hz), 4.23 (dd, 1H, *J* = 13.5 and 2.7 Hz), 5.00 (dd, 1H, *J* = 10.7 and 2.7 Hz), 5.34 and 5.51 (2 × s, 2H), 5.65 (dd, 1H, *J* = 7.3 and 2.8 Hz), 7.29-7.34 (m, 5H, Ar);
^{13}C NMR (75 MHz, $CDCl_3$) δ 21.52 (CH_2), 28.68 (CH_2), 43.43 (CH_2), 80.24 (CH), 90.84 (CH), 117.10 (CH_2), 126.50 (2 × CH), 128.29 (CH), 128.62 (2 × CH), 138.25 (C), 143.25 (C), 173.75 (C);
ESI-MS (m/z) 246 $[M+H]^+$; Anal. calcd for $C_{14}H_{15}NO_3$: C, 68.56%, H, 6.16%, N, 5.71%; found: C, 68.32%, H, 6.53%, N, 6.01%.

Compounds **5b-h** and **9a-h** were prepared from amides **3b-h** and **7a-h**, respectively using the same procedure.

In vitro Antimalarial assay: The *in vitro* antimalarial activity of the compounds was assessed against CQ-sensitive 3D7 strain of *P. falciparum* and compared with that of Chloroquine. The 50% inhibitory concentration (IC_{50}) were obtained following techniques of Smilkstein et al.[19] In brief the parasites were maintained in vitro in RPNI medium[20] supplemented with gentamycin at 40 µg/mL; (Sigma), Fungizone at 0.25 µg/mL; (GIBCO) and 10% fetal bovine serum (pH 7.2), at 37 ^{0}C in a CO_2 incubator.

The compounds were dissolved in DMSO at 5 mg/mL and required dilutions were made in a template plate with RPMI medium. 20 µL from each dilution was transferred, in duplicate, in the test plate and two wells receiving 20 µL of vehicle were kept as untreated control. For evaluation of IC_{50} of the compounds, SYBR Green I-based fluorescence (MSF) assay was used. For the assays, fresh dilutions of all compounds in screening medium were prepared and 50 µL of highest starting concentration (10–500 ng/mL) was dispensed in duplicate wells in row 'B' of 96-well tissue culture plate. The highest starting concentration for chloroquine was 25 ng/mL. Subsequently two fold serial dilutions were prepared up to row 'H' (seven concentrations) and finally 50 µL of 2.5% parasitized cell suspension containing 0.5% parasitaemia was added to each well except 4 wells in row 'A' received non-infected cell suspension. These wells containing non infected erythrocytes in the absence of compound served as negative control, while parasitized erythrocytes in the presence of CQ served as positive control. After incubating the plates for 72 h, 100 µL of lysis buffer [20 mM Tris (pH 7.5), 5 mM EDTA, 0.008% (wt/vol) saponin, and 0.08% (vol/vol) Triton X-100] containing 1 × concentration of SYBR Green-I was added to each well and incubated for 1 h at room temperature. The plates were examined for the relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLUOstar, BMG Labtechnologies). The IC_{50} was determined using Logit regression analysis of dose–response curves.

In vivo antimalarial efficacy test: The blood schizontocidal activity of the test compounds was evaluated in rodent model using multidrug-resistant strain of *Plasmodium yoelii nigeriensis*. The colony bred Swiss mice of either sex (20 ± 2 g) were inoculated intraperitoneally with 1×10^5 *P. yoelii* (MDR) parasites on day zero, and treatment was administered to group of five mice at each dose, from day 0 to 3, once daily. The drug dilutions of all compounds were prepared in groundnut oil so as to contain the required amount of the drug (1.2 mg/kg for a dose of 96 mg/kg) in 0.1 mL and administered orally for each dose. Parasitaemia level were recorded from thin blood smears on day 4 and subsequently twice a week till day 28. The animals which did not develop patent infection till day 28 were recorded as cured.[21] Mice treated with *β*-arteether served as positive control.

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[18] (a) 100% suppression of parasitaemia means no parasites were detected in 50 oil immersion microscopic fields (parasites if at all present, were below the detection limit). The parasites present below the detection limit can multiply and eventually can be detected during observation on subsequent days. In such cases though the drug is providing near 100% suppression of the parasitaemia on day 4 but will not provide full protection to the treated mice in the 28 day survival assay. (b) 100% protection means none of the treated mice developed patent infection during the 28 days observation period and hence were recorded as cured. Similarly 60% protection means only 3 out of 5 mice were cured.

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Synthesis and Antimalarial Activity of Novel Bicyclic and Tricyclic Aza-peroxides

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For the first time, novel bicyclic **5a-h** as well as tricyclic **9a-h** aza-peroxides were synthesized using $^1O_2$-mediated photo-oxygenation methodology as key step in 52-71% yields in which one of the oxygen atom of 1, 2, 4-trioxane ring has been replaced by nitrogen atom. The methodology is simple and is an efficient way to access 1,2-dioxa-4-aza six membered ring compounds. All these compounds were assessed for their *in vitro* antimalarial activity against *Plasmodium falciparum*. Compound **9a**, **9c** and **9f**, the most active compound of the series, showed IC_{50} values of 9.43, 8.83 and 5.63 ng/ml, respectively which was found to be comparable to that of antimalarial drug Chloroquine (IC_{50} value 5.2 ng/ml). Compound **9f**, the most active compound found in *in vitro* studies, provided 40% protection to the infected mice at the dose of 96 mg/kg × 4 days when screened for its antimalarial activity *in vivo* against multidrug-resistant *Plasmodium yoelii nigeriensis* in Swiss mice by oral route. In this assay, *β*-arteether and Chloroquine, showed 100% suppression of parasitaemia on day 4 and provided 100% and 80% protection, respectively, to the infected mice.