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A NEW CLASS OF POTENTIAL CHLOROQUINE RESISTANCE REVERSAL AGENTS FOR PLASMODIA: SYNTHESES AND BIOLOGICAL EVALUATION 1-[(3'-DIETHYLAMINOPROPYL)-3-(SUBSTITUTED PHENYLMETHYLENE)-PYRROLIDINES][#]

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Abstract: 1-[(3'-Diethylaminopropyl)-3-(substitutedphenylmethylene) pyrrolidines] were synthesized and evaluated for CQ resistant reversal activity. The compounds of the series elicit better biological response than their phenyl methyl analogues in general. The most active compound **4b** has been evaluated *in vivo* in details and the results are presented. The possible mode of action of the compounds of this series is by inhibition of the enzyme heme oxygenase, thereby increasing the levels of heme and hemozoin, which are lethal to the parasite.

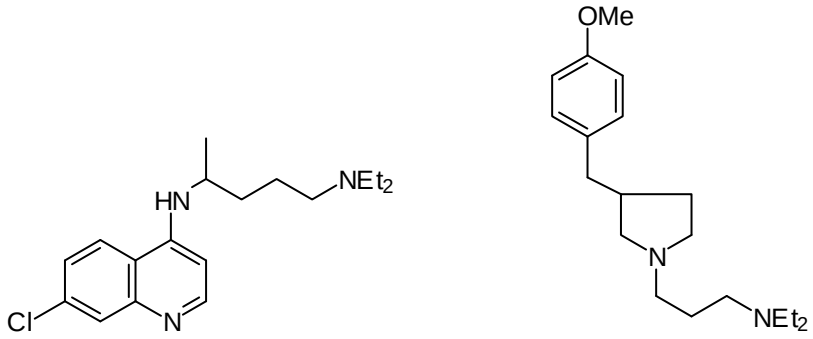
The disease malaria is caused by the protozoan parasite of the genus *Plasmodium* and it leads to mortality if subjects infected with *P. falciparum* are left untreated. This disease affects around 300 million subjects worldwide leading to more than 2 million deaths per year. The treatment of falciparum malaria has attracted global attention because of the growing resistance of the parasites towards all the known antimalarial drugs. Complete compliance of the recommended treatment by patients is desired for avoiding early development of the drug resistant strains of the parasite. In countries where hospital facilities cannot be extended to all patients, total compliance of recommended treatment is not assured. As an emergency measure, therefore, the most recent antimalarial drug such as artemisinin in these countries is recommended for hospital use only. The management for the control of this disease is further complicated by the absence of either any prophylactic vaccine or a new drug, which targets plasmodial genes.[1] The chemotherapy of malaria thus continues to be a matter of concern for medicinal chemists. Better clinical experience with the easily accessible antimalarial drug, chloroquine (CQ) (Fig. 1) does not help clinicians now because of the emergence of CQ resistant plasmodial strains. The present investigation, therefore, aims towards discovering compounds, which in combination with CQ, will make CQ resistant plasmodia susceptible to this combination therapy leading to the death of the resistant parasites. These compounds, hereafter for the sake of simplicity, will be addressed as CQ resistance reversal agents.[2] Search for such agents has led to biological evaluation of various calcium channel blockers,[3] antidepressants,[4] antihistamines[5] and prostaglandin oligomers[6] as CQ resistance reversal agents but they could not be pursued further since they were effective only *in vitro*.

The key point in designing the strategy towards the development of any such resistance reversal agent is the identification of the biochemical event, which may be correlated, with the

development of resistance. The biochemical event identified at our institute is increased capability of the CQ-resistant parasite to degrade heme or hemozoin through heme oxygenase associated with the increase in the glutathione-S-transferase activity.[7] Since it is almost certain that the heme-CQ complex is toxic to the parasite our effort towards the development of resistance reversal agent is concerned with the discovery of compounds, which may interfere with the heme degradation pathway of the CQ-resistant parasite. The earlier work from this laboratory have concluded that in comparison to CQ-sensitive parasite, the CQ-resistant parasite has enhanced heme oxygenase and decreased levels of heme/ hemozoin.[7] In another observation from our group it has been reported that heme oxygenase is responsible for heme and hemozoin degradation generated during the intra-erythrocytic development of malarial parasites of different species, viz. *P. falciparum, P. knowlesi, P. yoelii and P. berghei.*[8-9] In the light of these observations, it was envisaged that compounds capable of inhibiting heme degradation could increase the levels of heme-CQ complex and could thus cause the death of the CQ resistant parasites if given in combination with CQ. We have earlier reported a novel pyrrolidinoaminoalkane (CDRI 87/209) (Fig. 1), which was found to be CQ resistance reversal agent both *in vitro*[10-11] and *in vivo*.[12] In the study directed towards delineating its possible mode of action it has been observed that CDRI 87/209 showed inhibition against heme oxygenase and heme polymerase, the two enzymes involved in the heme degradation pathway. In our search for identification of newer molecular structures it was desired to synthesize compounds analogous to CDRI 87/209. The central point of designing new analogs was to bring restricted rotation around the position 3 of the pyrrolidine ring and then evaluate the biological activity. This led to the synthesis and evaluation of 1-[(3'-diethylaminopropyl)-3-(substituted phenylmethylene) pyrrolidines] as CQ resistance reversal agents. The details of this study are presented here.

Chemistry

The starting materials namely 3-substituted phenylmethylene-4, 5-dihydro-2 (3H)-furanones (**1**) used for the synthesis possible CQ resistance reversal agents, were obtained by the reacting



Chloroquine CDRI 87/209

Figure 1

substituted benzaldehyde with γ-butyrolactone in the presence of sodium methoxide. However, the compound **1d** was prepared by reaction of 4-benzyloxy benzaldehyde with γ-butyrolactone in the presence of sodium hydride in dry benzene. The next step of the scheme was to obtain diols (**2**) by reductive ring opening of the butyrolactone by slow addition of $LiAlH_4$ to compounds dissolved in dry ether under nitrogen. The diols were then subjected to mesylation in the presence of methanesulfonyl chloride to obtain the dimesyl derivative (**3**). These compounds on refluxing with primary amine in dry benzene cyclized to yield the pyrrolidines (**4**). Debenzylation of compound **4d** with sodium in liquid ammonia at -40^{o}C yielded the hydroxy derivative **5** in quantitative yield**.** This was alkylated with 1-(2-aminoethyl) pyrrolidine hydrochloride to yield the compound **6**. All the amines were converted to their oxalate salts by reacting them with oxalic acid in methanol-toluene mixture and adding dry ether precipitated them.

Chloroquine Resistance Reversal Activity. The parasitic infection was maintained by routinely inoculating 10^7 *P. berghei* K173 strain infected erythrocytes suspended in acid citrate dextrose solution using *M. coucha.* The CQ-resistant strain of *P. berghei* was developed by exposing the

sensitive strain infected animals to increasing doses of CQ and repassaging the infected blood into healthy animals. This was continued till the animals maintained a parasitaemia even after orally receiving 65 mg/kg b.wt. of CQ. The degree of drug pressure was periodically checked in infected mastomys. Simultaneously, *P. yoelii nigeriensis* infection was maintained in Swiss albino mice having CQ tolerance upto 120 mg/kg b. wt. Infection was ascertained by monitoring the Giemsa stained blood smears of infected animals microscopically. The isolation of the parasites was carried out from infected animal's blood through density gradient centrifugation and saponin lysis as described earlier. Heme oxygenase activity was assayed following the bilirubin formation in the post mitochondrial fraction. Different concentrations (10-100μM) of test compounds were supplemented in assay system and preincubated for 10 min. before starting the enzyme reaction.

In Vivo **Assay.** CQ/ compound **4b** was administered orally in aqueous medium to mastomys having 4 -5% parasitaemia of CQ-resistant *P. berghei* and mice having 1-2% parasitaemia of *P. yoelii nigeriensis* for 10 consecutive days. For studying the effect of compound **4b**, infected animals were divided into four groups of 10 individual each. The first group received CQ alone (10 mg/kg b. wt.); the second group received compound **4b** alone (15 mg/kg b. wt); the third group received a combination of compound **4b** and CQ (15 and 10 mg/kg b. wt, respectively) and the fourth group was kept as control without any treatment. The parasitaemia was monitored periodically. The feeding experiments of CQ/compound x were repeated with fresh batches of CQ-resistant ***P. berghei*** (5 times) or of *P. yoelii nigeriensis* (4 times) infected animals on different days, the cured mastomys/ mice blood was reinoculated into fresh normal animals for validation of resurgence of infection.

In a separate set of experiment, *P. yoelii nigeriensis* (multidrug resistant) and *P. berghei* (CQ resistant) infected mice and mastomys were orally fed compound **4b** (15 mg/kg b. wt.) or CQ (10 mg/kg b. wt.) for 6 and 10 days, respectively. After attainment of parasitaemia of about 70% in

former and about 25% in latter, the parasites were isolated and further processed for determination of heme oxygenase, heme polymerase, heme and hemozoin as reported earlier.[12]

Result and Discussion:

The main concern of the present investigation was to discover compounds capable of selective inhibition of heme degradation in plasmodia. Since the heme degradation is concerned with heme oxygenase activity, all the compounds were, therefore, screened to monitor the *in vitro* effect on heme oxygenase activity of cell free parasite *P. yoelii* as well as infected hepatic host enzyme for control. Amongst all the compounds screened *in vitro*, compounds **4c**, **4d**,and **6** were found to be complete inhibitors of parasite heme oxygenase but they did not inhibit the host enzyme while compounds **4a**, **4b** and **4e** inhibited both parasite and host heme oxygenase (Table 1). The K_I values of heme oxygenase with compound **4b** for CQ-resistant was found to be almost half of that of the CQ-sensitive strain of *P. berghei.* Since the main aim of this study was to find a CQ-resistance reversal agent which is active *in vivo* and has better activity profile than our earlier reported compound CDRI 87/209, all the compounds were first evaluated for their *in vivo* heme oxygenase inhibitory activity in *P. yoelii nigeriensis* isolated from infected mice. In this study, apart from heme oxygenase, the effect on another enzyme namely glutathione S-transferase was also monitored because the level of this enzyme was found to be higher in resistant plasmodia. Of all the compounds, **4b** showed complete inhibition of both the enzymes, while compounds **4c** and **6** exhibited potent inhibition but rest of the compounds did not show any inhibitory activity (Table 2). Based upon these observations the compound **4b** was selected for detailed *in vivo* evaluation for the CQ resistance reversal activity.

To evaluate the *in vivo* CQ resistance reversal effect of compound **4b**, it was screened against CQ-resistant *P. berghei* and multi drug resistant *P. yoelii nigeriensis* infected mastomys and mice, respectively to ascertain the susceptibility of plasmodia to CQ in the combination therapy. The

results in Tables 3 and 4 show the treatment schedule of CQ and the compound **4b**, percentage of parasitaemia at different time intervals and mean survival time of CQ-resistant *P. berghei* and multi drug resistant *P. yoelii nigeriensis* infected mastomys and mice, respectively. Dividing the infected animals in four groups followed the protocol of treatment. In the fourth group of mastomys having no treatment, the parasitaemia increased with increase in time, the maximum parasitaemia was about 30% on 20-22 day post infection, after which the animals started dying. All the treatments of CQ-resistant plasmodia in infected mice were started at 4-5% of parasitaemia. The first and the second group of animals received CQ and compound **4b** at 10 and 15 mg/kg b. wt., respectively for 10 consecutive days, and the parasitaemia developed similarly as in the control (fourth group). However, the third group of mastomys having received a combination of CQ and compound **4b** (10 and 15 mg/kg b. wt.) for 10 days shows the clearance of parasitaemia after about a week of cessation of treatment. It was also observed that there was no significant rise in parasitaemia during the schedule of treatment in this group. The survival time of the third group of mastomys was about three months (Table 3). Reinoculation of healthy animals with blood of the third group of mastomys did not show any recurrence of parasitaemia.

Similar to these observations parallel results were obtained when *P. yoelii nigeriensis* infected Swiss albino mice were used in place of *P. berghei* infected mastomys. However, the parasitaemia for starting the treatment schedule was 1-2%, the maximum parasitaemia attained by the animals was about 60-70% and the mean survival time was about a week in first, second and fourth groups of mice. The third group of mice receiving a combination therapy of CQ and compound **4b** (10 and 15 mg/kg b. wt.), respectively for 10 consecutive days survived for more than a month (Table 4).

In the study directed towards evaluation of status of heme oxygenase, heme and hemozoin in the parasites isolated from animals treated with compound **4b,** it was observed that CQ-resistant *P. berghei* has about ten times higher activity of heme oxygenase as compared to its CQ-sensitive counterpart. Heme and hemozoin levels showed about six and two folds decrease, respectively in the CQ-resistant plasmodia as compared to the CQ-sensitive strain (Table 5). The multi drug resistant *P. yoelii nigeriensis* isolated from the infected animals also showed complete inhibition of heme oxygenase and the levels of heme and hemozoin were increased and decreased, respectively (Table 6).

Conclusion. A new series of CQ resistance reversal agents was synthesized and evaluated. Prelimnary results indicate that of all the synthesized compounds, 1-[(3'-diethylaminopropyl)-3-((4-methoxyphenyl) methylene) pyrrolidines](**4b**) is the most active compound. The ability of this compound in combination with CQ to eliminate parasitaemia in experimental animals infected with the CQ-resistant plasmodia indicated the possibility of combating drug resistant plasmodia on the basis of envisaged concept delineated earlier in the text. This observation offers a new strategy of chemotherapy. However, more detailed study, particularly with drug resistant field isolates of *P. falciparum* is required before new drug developmental activities are initiated. The possible mode of action of compound **4b** relates to heme or hemozoin degradation pathway and more specifically on heme oxygenase of the CQ-resistant parasite thus increasing the concentration of heme in the food vacuole. This increased heme leads to enhanced levels of heme-CQ complex that is lethal to the parasite.

Experimental Section

Melting points are uncorrected and were determined in capillary tubes on a hot stage apparatus containing silicon oil. IR spectra were carried on Beckman Acculab-1spectrphotometer and ^{1}H NMR were recorded on Perkin Elmer R-32 or Bruker 400 FT NMR spectrometer, using TMS as

internal standard (chemical shifts in δ values, *J* in Hz). Mass spectrometry was carried out on a Jeol JMS-D-300 spectrometer. Elemental analyses were performed by a Carlo Erba 1108 microanalyzer and were within ±0.4% of calculated values in all cases.

General Procedure for Preparation of Diols (2a-e)

This procedure is illustrated for the preparation of 2-(phenylmethylene)-1,4-butanediol (**2a**). To an ice cooled stirred suspension of furanone **1a** (1.0 gm, 5.7mmol) in 150 mL of dry ether was added $LiAlH_4$ (0.2 gm, 5.5 mmol) in portions over a period of 10 min. under nitrogen. After the reaction mixture was stirred for 45 min. in an ice jacket, it was decomposed with chilled 1% aq. sodium hydroxide solution (10 mL) slowly. The precipitate was filtered and rejected. The organic layer of the filtrate was separated and dried over sodium sulfate. On evaporation, the pure diol was obtained in good yield and was used for further reaction without any purification (0.78gm, 76%): IR (neat) 3350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (t, 2H, *J*= 8Hz, CH_2), 3.55 (t, 2H, *J*= 8Hz, CH_2), 4.11 (s, 2H, CH_2), 6.53 (s, 1H, =CH), 7.19 (m, 5H, Ar-H); Mass (EI) m/z % 178(1.8). Anal ($C_{11}H_{14}O_2$) C, H.

2-[(4-methoxyphenyl) methylene)]-1,4-butanediol (2b). Similarly to the procedure described for **2a**, the title compound was prepared starting from **1b**. The product was obtained as a low melting solid (93%): IR (neat) 3400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (t, 2H, *J*= 8Hz, CH_2), 3.72 (m, 5H, CH_2 merged with Ar-OCH_3), 4.10 (s, 2H, CH_2), 6.45 (s, 1H, =CH), 6.75 (d, 2H, *J*= 9Hz, Ar-H), 7.10 (d, 2H, *J*= 9Hz, Ar-H); Mass (EI) m/z % 208 (18.9). Anal ($C_{12}H_{16}O_3$) C, H.

2-[(3,4-dimethoxyphenyl) methylene)]-1,4-butanediol (2c). Similarly to the procedure described for **2a**, the title compound was prepared starting from **1c**. The product was obtained as an oil (85%): IR (neat) 3400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.57 (t, 2H, *J*= 8Hz, CH_2), 3.55 (t, 2H *J*= 8Hz, CH_2), 3.82 (s, 6H, 2 X Ar-OCH_3), 4.12 (s, 2H, CH_2), 6.47 (s, 1H, =CH), 6.75 (m, 2H, Ar-H), 6.92 (m, 1H, Ar-H); Mass (EI) m/z % 238 (21.7). Anal ($C_{13}H_{18}O_4$) C, H.

2-[(3,4-methylenedioxyphenyl) methylene)]-1,4-butanediol (2d). Similarly to the procedure described for **2a**, the title compound was prepared starting from **1d**. The product was obtained as an oil (74%): IR (neat) 3400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51(t, 2H, *J*= 8Hz, CH_2), 3.58 (t, 2H *J*= 8Hz, CH_2), 4.08 (s, 2H, CH_2), 5.81 (s, 2H, OCH_2O), 6.47 (s, 1H, =CH), 6.75 (m, 2H, Ar-H), 6.92 (m, 1H, Ar-H); Mass (EI) m/z % 222 (16.8). Anal ($C_{12}H_{14}O_4$) C, H.

2-[(4-benzyloxyphenyl) methylene)]-1,4-butanediol (2e). Similarly to the procedure described for **2a**, the title compound was prepared starting from **1e**. The product was obtained as a solid (74%): m.p. 89^oC; IR (neat) 3320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.61 (t, 2H, *J*= 8Hz, CH_2), 3.79 (t, 2H *J*= 8Hz, CH_2), 4.19 (s, 2H, CH_2), 5.04 (s, 2H, CH_2Ph), 6.53 (s, 1H, =CH), 7.09 (m, 9H, Ar-H; Mass (EI) m/z % 284 (17.8). Anal ($C_{18}H_{20}O_3$) C, H.

General Procedure for Preparation of Dimesylates (3a-e)

This procedure is illustrated for the preparation of 2-[(4-benzyloxyphenyl) methylene)]-1,4-dimethanesulfonyloxybutane (**3e)**. To an ice cooled stirred solution of diol (2.5 gm, 8.8mmol) in 80 mL dry benzene were simultaneously added dropwise a solution of triethylamine (2.46mL, 17.6 mmol) and methanesulfonyl chloride (1.37mL, 17.6mmol) in dry benzene. The reaction was allowed to stir at 0-5^oC for 6 h. On completion the reaction mixture was extracted with water. The organic layer was separated and dried over sodium sulfate. The evaporation of solvent furnished the dimesyl derivative (**3e**) as an oil (yield 3.3gm, 96%). Since these dimesylates were not very stable they were immediately utilized for further reaction: ^1H NMR ($CDCl_3$) δ 1.92 (t, 2H, CH_2), 2.95 (s, 6H, 2 X SO_2CH_3), 4.22 (m, 4H, 2X-CH_2), 4.99 (s, 2H, CH_2-Ph), 7.14 (m, 10H, Ar-H & =CH); MS (EI) m/z % 440 (11.2). Anal ($C_{20}H_{24}S_2O_7$) N.D.

General Procedure for Preparation of Pyrrolidines (4a-e)

This procedure is illustrated for the preparation of 3-[(4-methoxyphenyl) methylene]-1-(3-N, N-diethylaminopropyl) pyrrolidine (**4b)**. A mixture of dimesylate **3b** (2.0 gm, 5mmol), N, N'-diethyl-aminopropylamine (4.8mL, 30mmol) and triethylamine (2.8mL, 20mmol) in 50mL of dry benzene was heated at reflux with stirring for 10 h. The solvent was evaporated, and the residue was partitioned between water and ethylacetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The residue was column chromatographed over basic alumina using chloroform: methanol (99:1, v/v) as eluent to yield the pyrrolidine (**4b)** in 57% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.70 (m, 4H, 2XCH_2), 2.53 (m, 10H, 5 X CH_2), 3.20 (s, 2H, CH_2), 3.73 (s, 3H, Ar-OCH_3), 6.19 (brs, 1H, =CH), 6.88 (d, 2H, *J*= 8Hz, Ar-H), 7.16 (d, 2H, *J*= 8Hz, Ar-H); MS (EI) m/z % 302 (18.1).

General Procedure for Preparation of Oxalate salts of Pyrrolidines

This procedure is illustrated for the preparation of oxalate salt of compound **4b**. To the solution of compound **4b** (3.0 gm) in dry methanol (5 mL) was added a solution of oxalic acid dihydrate (1.25gm) in dry methanol (10mL). The mixture was hand shaked for 10 min. and then dry ether was added freely to precipitate the salt. This crude salt was recrystallized from dry methanol (2.54gm, 53%): m.p. 184-85^{o}C. Anal. [$C_{19}H_{30}N_2O$. 2$(CO_2H)_2$] C, H, N.

3-(phenylmethylene)-1-(3-N, N-diethylaminopropyl) pyrrolidine (4a) Similarly to the procedure described for **4b**, the title compound was prepared starting from **1a**. The product was obtained as an oil (62%): ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.70 (m, 4H, 2 X CH_2), 2.53 (m, 10H, 5 X CH_2), 3.29 (s, 2H, CH_2), 6.28 (brs, 1H, =CH), 7.22 (m, 5H, Ar-H); MS (EI) m/z % 272 (20.9). **Oxalate salt**: 143-44 ^{o}C; Anal. [$C_{18}H_{28}N_2$.2 $(CO_2H)_{2]}$ C, H, N.

3-[(3,4-dimethoxyphenyl) methylene]-1-(3-N, N-diethylaminopropyl) pyrrolidine (4c) Similarly to the procedure described for **4b**, the title compound was prepared starting from **1c**. The product was

obtained as an oil (65%): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.72 (m, 4H, 2 X CH_2), 2.52 (m, 10H, 5 X CH_2), 3.31(s, 2H, CH_2), 3.89 (s, 6H, 2 X Ar-OCH_3), 6.28 (brs, 1H, =CH), 6.75 (m, 2H, Ar-H), 6.84 (m, 1H, Ar-H); MS (EI) m/z % 332 (21.9). **Oxalate salt**: 143-44 ^{o}C; Anal. [$C_{20}H_{32}N_2O_2$. 2$(CO_2H)_2$] C, H, N.

3-[(3,4-methylenedioxyphenyl) methylene]-1-(3-N, N-diethylaminopropyl) pyrrolidine (4d) Similarly to the procedure described for **4b**, the title compound was prepared starting from **1d**. The product was obtained as an oil (59%): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.68 (m, 4H, 2 X CH_2), 2.58 (m, 10H, 5 X CH_2), 3.31(s, 2H, CH_2), 5.87 (s, 2H, OCH_2O), 6.28 (brs, 1H, =CH), 6.44 (m, 2H, *J*= 8Hz, Ar-H), 6.81 (m, 2H, *J*= 8Hz, Ar-H); MS (EI) m/z % 316 (21.9). **Oxalate salt**: 167-69 ^{o}C; Anal. [$C_{20}H_{32}N_2O_2$. 2$(CO_2H)_2$] C, H, N.

3-[(4-benzyloxyphenyl) methylene]-1-(3-N, N-diethylaminopropyl) pyrrolidine (4e) Similarly to the procedure described for **4b**, the title compound was prepared starting from **1e**. The product was obtained as an oil (52%): ^{1}H NMR ($CDCl_3$) δ 1.02 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.43 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 2.56 (m, 10H, 5 X CH_2), 3.26(s, 2H, CH_2), 4.99 (s, 2H, CH_2Ph), 6.23 (brs, 1H, =CH), 7.11 (m, 9H, Ar-H); MS (EI) m/z % 378 (13.2). **Oxalate salt**: 141-42 ^{o}C. Anal. [$C_{25}H_{34}N_2O$. 2$(CO_2H)_2$] C, H, N.

Typical procedure for the preparation of compound 5

Ammonia was first condensed in a round bottom flask and was allowed to distill through a passing tube into another flask containing the compound **4e** (2.0 gm, 5.3mmol) in 30 mL of dry ether. Small amount of sodium chips was then added at a temperature between –40^o C and –45^{o}C till the reaction mixture turns blue black. The reaction was stirred at the same temperature for 45 min. On completion, the reaction mixture was allowed to come to room temperature (30^{o}C) and then solid ammonium chloride was added till the solution becomes clear. Thereafter, the ammonia was boiled

off and the solution was filtered. The filtrate was evaporated to give the hydroxy derivative as an oil (1.48 gm, 98%): IR (neat) 3380 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.53 (m, 4H, 2 X CH_2), 2.49 (m, 10H, 5 X CH_2), 3.24 (s, 2H, CH_2), 6.32 (brs, 1H, =CH), 6.83(m, 4H, Ar-H); MS (EI) m/z % 288 (33). Anal. ($C_{18}H_{28}N_2O$) N.D.

Typical procedure for the preparation of compound 6

To a stirred solution of compound **5** (0.5 gm, 1.73mmol) in 30 mL of dry acetone were added baked potassium carbonate (0.4 gm, 6.8mmol) and N, N'-diethylaminoethylpyrrolidine hydrochloride (0.35 gm, 2 mmol). The reaction mixture was refluxed for 20 h and after cooling the inorganic material was filtered and the filtrate was evaporated to furnish a residue. This oil upon chromatography over basic alumina using methanol: chloroform (1:99, v/v) as an eluent furnished the 3-[(4-(2-pyrrolidin-1-yl) methoxyphenyl) methylene]-1-(3-N, N-diethylaminopropyl) pyrrolidine as an oil (0.45 gm, 68%): ^1H NMR ($CDCl_3$) δ 1.02 (t, 6H, *J*= 8Hz, 2 X CH_3), 1.78 (m, 8H, 4 X CH_2), 2.58-(m, 14H, 7 X CH_2), 2.88 (t, 2H, CH_2), 3.42(s, 2H, CH_2), 4.11 (t, 2H, CH_2), 6.40 (brs, 1H, =CH), 6.88 (d, 2H, *J*= 8Hz, Ar-H), 7.11 (d, 2H, *J*= 8Hz, Ar-H); MS (EI) m/z % 385 (43.2). **Oxalate salt**: 118-21 ^oC. Anal. [$C_{25}H_{34}N_2O.\ H_2O.\ 3(CO_2H)_2$] C, H, N.

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Table 1*: In vitro* effect of different compounds on heme oxygenase activity of cell free parasite *P. yoelii* and corresponding infected host

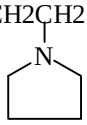
R	Compound no.	Concentration (μM)	% inhibition in heme oxygenase	
			Parasite	Host
H	**4a**	10	52	10
		50	64	65
		100	82	65
4-OMe	**4b**	10	48	Nil
		50	64	21
		100	87	31
3,4-$(OMe)_2$	**4c**	10	100	Nil
		50	100	Nil
		100	100	Nil
3,4-OCH_2O	**4d**	10	100	Nil
		50	100	Nil
		100	100	Nil
4-OCH_2Ph	**4e**	10	28	24
		50	37	63
		100	64	69
4- 	**6**	10	100	Nil
		50	100	Nil
		100	100	Nil
-	Chlorpromazine	50	85	Nil
		100	100	Nil
-	Diltiazem	50	12.5	41
		100	29	84
-	Verapamil	50	100	15
		100	100	19

Table 2. Status of heme oxygenase and glutathione S-transferase activities in *P. yoelii nigeriensis* isolated from infected mice orally treated with the compounds

Compound (15 mg/kg b. wt. for 5 days)	% inhibition in Heme oxygenase	Glutathione S-transferase
4a	Nil	Nil
4b	100	99
4c	100	80
4d	Nil	10
4e	Nil	Nil
6	79	90

Table 3*: In vivo* evaluation of compound 4b as resistance reversal agent in CQ-resistant *P. berghei* infected *Masotomys coucha*

Parameter	Group 1	Group II	Group III	Group IV
Parasitaemia before treatment	4-5%	4-5%	4-5%	4-5%
Dose of CQ or/& Compound **4b**	CQ: 10mg/kg b. wt.	Compd **4b**: 15 mg/kg b. wt	CQ: 10mg & Compd **4b** 15 mg/kg b. wt	Nil
Period of treatment	10 days	10 days	10 days	Not applicable
Parasitaemia on 10^{th} day of treatment	15-17%	20-22%	Nil	20-22%
Parasitaemia a week after a 10 day treatment	>25-30%	>25-30%	Nil	>25-30%
Mean survival Time	20-22 days	20-22 days	Observed for 3 months, no death	20-22 days

Each group constitutes 20 mastomys infected with CQ-resistant P. berghei k 173 strain (able to tolerate 65-70 mg/kg b. wt. of CQ base orally; sensitive strain could tolerate 8 mg/kg b. wt. The experiment was repeated 5 times. CQ and compound were fed orally in aqueous solution.

Table 4. *In vivo* evaluation of compound **4b** as resistance reversal agent in multidrug resistant *P. yoelii nigerienses* infected Swiss albino mice

Parameters	Group 1	Group II	Group III	Group IV
Parasitaemia before Treatment	1-2%	1-2%	1-2%	1-2%
Dose of CQ or/& Compound **4b**	CQ: 10mg/kg b. wt.	Compd **4b** : 15 mg/kg b. wt	CQ: 10mg & Compd. **4b** 15 mg/kg b. wt	Nil
Period of treatment	6 days	6 days	10 days	Not applicable
Parasitaemia on 6^{th}/ 10^{th} day of treatment	70-80%	70-80%	Nil	70-80%
Parasitaemia a week after a 10 day treatment	No survival	No survival	Nil	No survival
Mean survival Time	One week	One week	Observed for 1 month, no death	One week

Each group constitutes 20 mice infected with multidrug resistant P. yoelii nigerienses (able to tolerate 120 mg/kg b. wt. of CQ base orally).

Table 5. Status of heme oxygenase, heme and hemozoin in CQ sensitive, CQ resistant and CQ-resistant *P. berghei* treated with compound **4b** (15 mg/kg b. wt.) for 10 days.

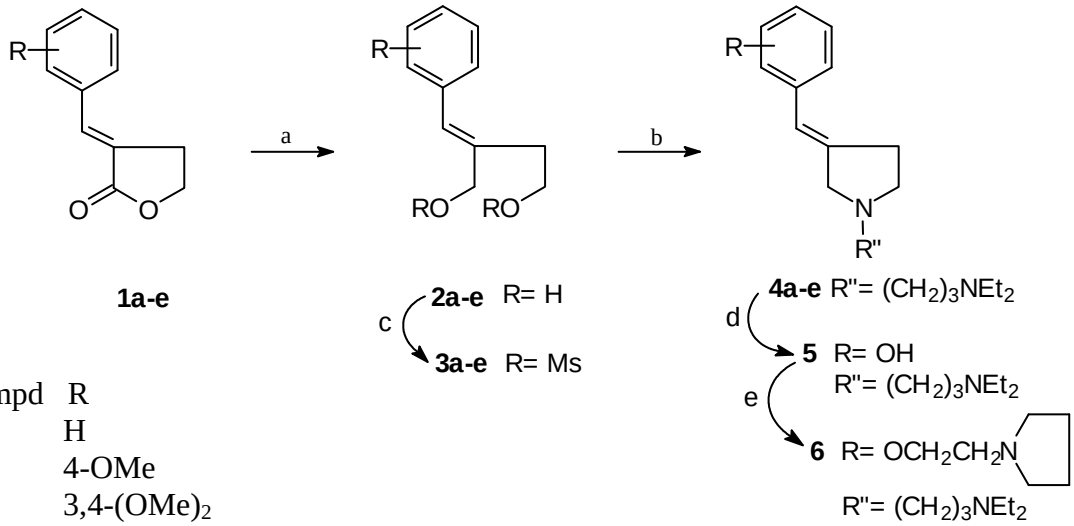
Samples	Heme oxygenase *	Heme**	Hemozoin**
CQ sensitive *P. berghei*	7.32 ± 0.71	344 ± 12.17	101.36 ± 10.13
CQ resistant *P. berghei*	80.17 ± 14.24	60.29 ± 9.32	51.39 ± 7.18
CQ resistant *P. berghei* treated with Compound **4b**	No activity	321.13 ± 24.86	71.14 ± 13.32[a]

*Values are mean ± SD of 4 separate observations. Sp. activities in * n moles/ hr/ mg protein, ** p moles/ mg protein. P< 0.001, [a] P=NS*

Table 6. Status of heme oxygenase, heme and hemozoin in CQ-sensitive, CQ-resistant and CQ-resistant *P. yoelii nigeriensis* treated with compound **4b** (15 mg/kg b. wt.) for 10 day

Samples	Heme oxygenase *	Heme**	Hemozoin**
CQ sensitive *P. yoelii*	10.23 ± 1.81	218.13 ± 15.64	109.18 ± 17.14
CQ resistant *P. yoelii*	101.86 ± 13.14	109.31 ± 12.13	43.29 ± 13.74
CQ resistant *P. yoelii* treated with Compound **4b**	No activity	172.14 ± 19.86	45.15 ± 12.62[a]

*Values are mean ± SD of 4 separate observations. Sp. activities in * n moles/ hr/ mg protein, ** p moles/mg protein. P<0.001, [a]P=NS.*



Compd	R
a	H
b	4-OMe
c	3,4-$(OMe)_2$
d	3,4-OCH_2O
e	4-OCH_2Ph

Scheme 1: a) $LiAlH_4$, dry ether; b) MsCl, Et_3N, dry benzene; c) N,N'-diethylaminopropylamine, Et_3N, dry benzene; d) Na, liquid NH_3, dry ether; e) 2-(pyrrolidin-1-yl)ethylchloride hydrochloride, K_2CO_3, dry acetone.

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Chemical Analyses of Compounds

Compound No.	Calculated	Found
2a	C: 74.13, H: 7.92	C: 74.41, H: 7.86
2b	C: 69.22, H: 5.43	C: 68.96, H: 5.23
2c	C: 6.94, H: 5.62	C: 66.88, H: 5.68
2d	C: 64.85, H: 7.14	C: 65.06, H: 6.99
2e	C: 76.03, H: 7.08	C: 75.89, H: 7.25
4a	C: 57.23, H: 6.89, N: 6.38	C: 57.32, H: 6.79, N: 6.76
4b	C: 57.25, H: 7.10, N: 5.80	C: 56.91, H: 7.39, N: 5.70
4c	C: 56.24, H: 7.07, N: 5.46	C: 56.49, H: 7.40, N: 5.49
4d	C: 53.48, H: 7.03, N: 6.23	C: 53.48, H: 7.03, N: 6.23
4e	C: 62.35, H: 6.85, N: 5.01	C: 62.53, H: 7.19, N: 5.37
6	C: 53.48, H: 7.03, N: 6.23	C: 53.12, H: 6.77, N: 5.83