# A New Class of Potential Chloroquine-Resistance Reversal Agents for Plasmodia: Syntheses and Biological Evaluation of 1-(3′-Diethylaminopropyl)-3-(substituted phenylmethylene)pyrrolidines[#]

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1-(3′-Diethylaminopropyl)-3-(substituted phenylmethylene)pyrrolidines were synthesized and evaluated for CQ-resistant reversal activity. In general the compounds of the series elicit better biological response than their phenylmethyl analogues. The most active compound **4b** has been evaluated in vivo in detail, 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.

## Introduction

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 toward 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), does not help clinicians now because of the emergence of CQ-resistant plasmodial strains. The present investigation, therefore, aims toward 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



Chloroquine          CDRI 87/209
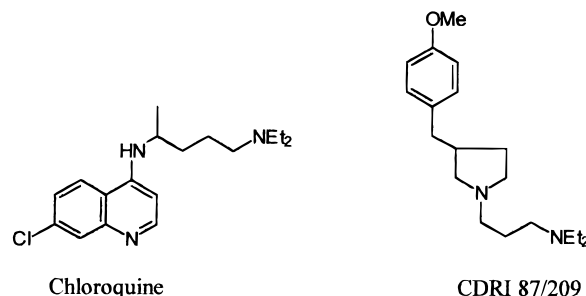
such agents has led to biological evaluation of various calcium channel blockers,[3] antidepressants,[4] antihistamines,[5] and prostaglandin oligomers[6] as CQ-resistance reversal agents, but they could not be pursued further since they were effective only in vitro.

The key point in designing the strategy toward 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 an increase in glutathione-S-transferase activity.[7] Since it is almost certain that the heme−CQ complex is toxic to the parasite, our effort toward the development of a resistance reversal agent is concerned with the discovery of compounds that may interfere with the heme degradation pathway of the CQ-resistant parasite. The earlier works from this laboratory have concluded that in comparison to the 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 light of these observations, it was envisaged that compounds capable of inhibiting heme degradation could increase the levels of the heme−CQ complex and could thus cause the death of the CQ-resistant parasites

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**Scheme 1**[a]



| Compd | R |
|---|---|
| a | H |
| b | 4-OMe |
| c | 3,4-(OMe)$_2$ |
| d | 3,4-OCH$_2$O |
| e | 4-OCH$_2$Ph |
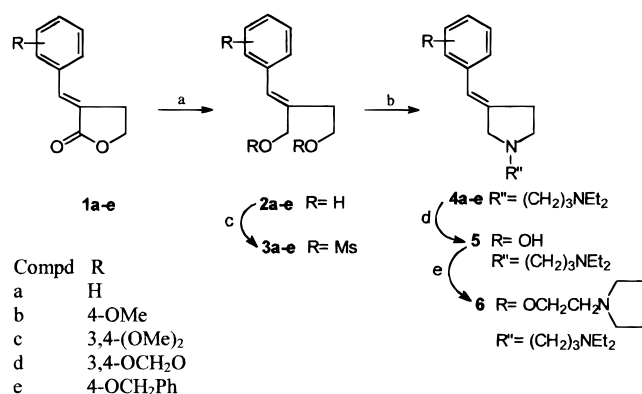
[a] (a) LiAlH$_4$, dry ether; (b) MsCl, Et$_3$N, dry benzene; (c) *N,N*-diethylaminopropylamine, Et$_3$N, dry benzene; (d) Na, liquid NH$_3$, dry ether; (e) 2-(pyrrolidin-1-yl)ethyl chloride hydrochloride, K$_2$CO$_3$, dry acetone.

if given in combination with CQ. We have earlier reported a novel pyrrolidinoaminoalkane (CDRI 87/209), which was found to be a CQ-resistance reversal agent both in vitro[10,11] and in vivo.[12] In the study directed toward 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 analogues 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(3*H*)-furanones **1** (Scheme 1), used for the synthesis of possible CQ-resistance reversal agents were obtained by reacting substituted benzaldehyde with γ-butyrolactone in the presence of sodium methoxide. However, compound **1d** was prepared by reaction of 4-benzyloxybenzaldehyde with γ-butyrolactone in the presence of sodium hydride in dry benzene. The next step 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 pyrrolidines **4**. Debenzylation of compound **4d** with sodium in liquid ammonia at −40 °C yielded the hydroxy derivative **5** in quantitative yield. This was alkylated with 1-(2-aminoethyl)pyrrolidine hydrochloride to yield compound **6**. All the amines were converted to their oxalate salts by reacting them with oxalic acid in a methanol–toluene mixture and then adding dry ether to precipitate them.

**CQ-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 parasitemia even after orally receiving 65 mg/kg body 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 up to 120 mg/kg body 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 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 (10−100 μM) of test compounds were supplemented in the 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% parasitemia of CQ-resistant *P. berghei* and mice having 1−2% parasitemia of *P. yoelii nigeriensis* for 10 consecutive days. For studying the effect of compound **4b**, infected animals were divided into 4 groups of 10 mice each. The first group received CQ alone (10 mg/kg body wt); the second group received compound **4b** alone (15 mg/kg body wt); the third group received a combination of compound **4b** and CQ (15 and 10 mg/kg body wt, respectively); the fourth group was kept as a control without any treatment. The parasitemia was monitored periodically. The feeding experiments of CQ/compound **4b** were repeated with fresh batches of CQ-resistant *P. berghei* (5 times) or *P. yoelii nigeriensis* (4 times) infected animals on different days, and the cured mastomys/mice blood was reinoculated into fresh normal animals for validation of resurgence of infection.

In a separate set of experiments, *P. yoelii nigeriensis* (multidrug-resistant) and *P. berghei* (CQ-resistant) infected mice and mastomys were orally fed compound **4b** (15 mg/kg body wt) or CQ (10 mg/kg body wt) for 6 and 10 days, respectively. After attainment of parasitemia 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]

## Results and Discussion

The main concern of the present investigation was to discover compounds capable of selective inhibition of heme degradation in plasmodia. Since heme degradation involves 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. Among 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

**Table 1.** In Vitro Effect of Different Compounds on Heme Oxygenase Activity of Cell-Free Parasite *P. yoelii* and the Corresponding Infected Host

| | | | % inhib in heme oxygenase | |
|---|---|---|---|---|
| R | compd | concn ($\mu$M) | 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$_2$O | **4d** | 10 | 100 | nil |
| | | 50 | 100 | nil |
| | | 100 | 100 | nil |
| 4-OCH$_2$Ph | **4e** | 10 | 28 | 24 |
| | | 50 | 37 | 63 |
| | | 100 | 64 | 69 |
| 4-OCH2CH2 (N, pyrrolidine ring) | **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

| | % inhibition in | |
|---|---|---|
| compd (15 mg/kg body wt for 5 days) | heme oxygenase | glutathione-S-transferase |
| **4a** | nil | nil |
| **4b** | 100 | 99 |
| **4c** | 100 | 80 |
| **4d** | nil | 10 |
| **4e** | nil | nil |
| **6** | 79 | 90 |

compound **4b** for CQ-resistant was found to be almost one-half 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 a 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; the remaining compounds did not show any inhibitory activity (Table 2). Upon the basis of these observations compound **4b** was selected for detailed in vivo evaluation for 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 multidrug-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 compound **4b**, percentage of parasitemia at different time intervals, and mean survival time of CQ-resistant *P. berghei* and multidrug-resistant *P. yoelii nigeriensis* infected mastomys and mice, respectively. Dividing the infected animals into four groups followed the protocol of treatment. In the fourth group of mastomys having no treatment, the parasitemia increased with an increase in time and the maximum parasitemia 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% parasitemia. The first and second groups of animals received CQ and compound **4b** at 10 and 15 mg/kg body wt, respectively, for 10 consecutive days, and the parasitemia 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 body wt) for 10 days showed clearance of parasitemia after about 1 week after cessation of treatment. It was also observed that there was no significant rise in parasitemia during the schedule of treatment in this group. The survival time of the third group of mastomys was about 3 months (Table 3). Reinoculation of healthy animals with blood of the third group of mastomys did not show any recurrence of parasitemia.

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 parasitemia for starting the treatment schedule was 1–2%, the maximum parasitemia attained by the animals was about 60–70%, and the mean survival time was about 1 week in the 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 body wt, respectively) for 10 consecutive days survived for more than 1 month (Table 4).

In the study directed toward 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 10 times higher activity of heme oxygenase as compared to its CQ-sensitive counterpart. Heme and hemozoin levels showed about 6- and 2-fold decreases, respectively, in the CQ-resistant plasmodia as compared to the CQ-sensitive strain (Table 5). The multidrug-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. Preliminary results indicate that of all the synthesized compounds, 1-(3′-diethylaminopropyl)-3-((4-methoxyphenyl)methylene)pyrrolidine (**4b**) is the most active compound. The ability of this compound in combination with CQ to eliminate parasitemia in experimental animals infected with the CQ-resistant plasmodia indicated the possibility of combating drug-resistant plasmodia on the basis of the envisaged concept delineated earlier in the text. This observation offers a new strategy for chemotherapy. However, more detailed studies, particularly with drug-

**Table 3.** In Vivo Evaluation of Compound **4b** as a Resistance Reversal Agent in CQ-Resistant *P. berghei* Infected *M. coucha*[a]

| parameter | group I | group II | group III | group IV |
|---|---|---|---|---|
| parasitemia before treatment | 4−5% | 4−5% | 4−5% | 4−5% |
| dose of CQ and/or compound **4b** | CQ: 10 mg/kg body wt | **4b**: 15 mg/kg body wt | CQ: 10 mg/kg body wt and **4b**: 15 mg/kg body wt | nil |
| period of treatment | 10 days | 10 days | 10 days | not applicable |
| parasitemia on day 10 of treatment | 15−17% | 20−22% | nil | 20−22% |
| parasitemia 1 week after 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 |

[a] Each group constituted 20 mastomys infected with CQ-resistant *P. berghei* K173 strain (able to tolerate 65−70 mg/kg body wt of CQ base orally; sensitive strain could tolerate 8 mg/kg body 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 a Resistance Reversal Agent in Multidrug-Resistant *P. yoelii nigerienses* Infected Swiss Albino Mice[a]

| parameter | group I | group II | group III | group IV |
|---|---|---|---|---|
| parasitemia before treatment | 1−2% | 1−2% | 1−2% | 1−2% |
| dose of CQ and/or compound **4b** | CQ: 10 mg/kg body wt | **4b**: 15 mg/kg body wt | CQ: 10 mg/kg body wt and **4b**: 15 mg/kg body wt | nil |
| period of treatment | 6 days | 6 days | 10 days | not applicable |
| parasitemia on day 6/10 of treatment | 70−80% | 70−80% | nil | 70−80% |
| parasitemia 1 week after 10-day treatment | no survival | no survival | nil | no survival |
| mean survival time | 1 week | 1 week | observed for 1 month, no death | 1 week |

[a] Each group constituted 20 mice infected with multidrug-resistant *P. yoelii nigerienses* (able to tolerate 120 mg/kg body 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 body wt) for 10 Days

| sample | heme oxygenase[a] | heme[b] | hemozoin[b] |
|---|---|---|---|
| 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 **4b** | no activity | 321.13 ± 24.86 | 71.14 ± 13.32[c] |

[a] Values are mean ± SD of 4 separate observations. Specific activity in nmol/h/mg protein. [b] Specific activity in pmol/mg protein. $P < 0.001$. [c] $P$ = 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 body wt) for 10 Days

| sample | heme oxygenase[a] | heme[b] | hemozoin[b] |
|---|---|---|---|
| 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 **4b** | no activity | 172.14 ± 19.86 | 45.15 ± 12.62[c] |

[a] Values are mean ± SD of 4 separate observations. Specific activity in nmol/h/mg protein. [b] Specific activity in pmol/mg protein. $P < 0.001$. [c] $P$ = NS.

resistant field isolates of *P. falciparum,* are required before new drug developmental activities are initiated. The possible mode of action of compound **4b** relates to the heme or hemozoin degradation pathway and more specifically to 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 the 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 out on a Beckman Acculab-1 spectrphotometer and $^1$H NMR were recorded on a Perkin-Elmer R-32 or Bruker 400 FT NMR spectrometer, using TMS as internal standard (chemical shifts in $\delta$ 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-(phenylmeth-

ylene)-1,4-butanediol (**2a**). To an ice-cooled stirred suspension of furanone **1a** (1.0 g, 5.7 mmol) in 150 mL of dry ether was added LiAlH$_4$ (0.2 g, 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 (0.78 g, 76%): IR (neat) 3350 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 2.55 (t, 2H, $J$ = 8 Hz, CH$_2$), 3.55 (t, 2H, $J$ = 8 Hz, CH$_2$), 4.11 (s, 2H, CH$_2$), 6.53 (s, 1H, =CH), 7.19 (m, 5H, Ar−H); MS (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$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 2.54 (t, 2H, $J$ = 8 Hz, 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, $J$ = 9 Hz, Ar−H), 7.10 (d, 2H, $J$ = 9 Hz, Ar−H); MS (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$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 2.57 (t, 2H, $J = 8$ Hz, CH$_2$), 3.55 (t, 2H, $J = 8$ Hz, CH$_2$), 3.82 (s, 6H, 2 × 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); MS (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$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 2.51 (t, 2H, $J = 8$ Hz, CH$_2$), 3.58 (t, 2H, $J = 8$ Hz, CH$_2$), 4.08 (s, 2H, CH$_2$), 5.81 (s, 2H, OCH$_2$O), 6.47 (s, 1H, =CH), 6.75 (m, 2H, Ar−H), 6.92 (m, 1H, Ar−H); MS (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%): mp 89 °C; IR (neat) 3320 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 2.61 (t, 2H, $J = 8$ Hz, CH$_2$), 3.79 (t, 2H, $J = 8$ Hz, CH$_2$), 4.19 (s, 2H, CH$_2$), 5.04 (s, 2H, CH$_2$Ph), 6.53 (s, 1H, =CH), 7.09 (m, 9H, Ar−H); MS (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 g, 8.8 mmol) in 80 mL dry benzene were simultaneously added dropwise a solution of triethylamine (2.46 mL, 17.6 mmol) and methanesulfonyl chloride (1.37 mL, 17.6 mmol) in dry benzene. The reaction was allowed to stir at 0−5 °C 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 3.3 g, 96%). Since these dimesylates were not very stable they were immediately utilized for further reaction: $^1$H NMR (CDCl$_3$) $\delta$ 1.92 (t, 2H, CH$_2$), 2.95 (s, 6H, 2 × SO$_2$CH$_3$), 4.22 (m, 4H, 2 × −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$_2$O$_7$) 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 g, 5 mmol), *N,N*-diethylaminopropylamine (4.8 mL, 30 mmol) and triethylamine (2.8 mL, 20 mmol) in 50 mL of dry benzene was heated at reflux with stirring for 10 h. The solvent was evaporated, and the residue was partitioned between water and ethyl acetate. 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$_3$) $\delta$ 0.90 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.70 (m, 4H, 2 × CH$_2$), 2.53 (m, 10H, 5 × CH$_2$), 3.20 (s, 2H, CH$_2$), 3.73 (s, 3H, Ar−OCH$_3$), 6.19 (brs, 1H, =CH), 6.88 (d, 2H, $J = 8$ Hz, Ar−H), 7.16 (d, 2H, $J = 8$ Hz, 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 the oxalate salt of compound **4b**. To the solution of compound **4b** (3.0 g) in dry methanol (5 mL) was added a solution of oxalic acid dihydrate (1.25 g) in dry methanol (10 mL). 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 (2.54 g, 53%): mp 184−85 °C. Anal. [C$_{19}$H$_{30}$N$_2$O·2(CO$_2$H)$_2$] 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$_3$) $\delta$ 0.98 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.70 (m, 4H, 2 × CH$_2$), 2.53 (m, 10H, 5 × 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: mp 143−44 °C. Anal. [C$_{18}$H$_{28}$N$_2$·2(CO$_2$H)$_2$] C, H, N.

**3-[(3,4-Dimethoxyphenyl)methylene]-1-(3-*N,N*-diethylaminopropyl)pyrrolidine (4c).** Similarly to the procedure described for

described for **4b**, the title compound was prepared starting from **1c**. The product was obtained as an oil (65%): $^1$H NMR (CDCl$_3$) $\delta$ 1.04 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.72 (m, 4H, 2 × CH$_2$), 2.52 (m, 10H, 5 × CH$_2$), 3.31 (s, 2H, CH$_2$), 3.89 (s, 6H, 2 × 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: mp 143−44 °C. Anal. [C$_{20}$H$_{32}$N$_2$O$_2$·2(CO$_2$H)$_2$] 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$_3$) $\delta$ 1.04 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.68 (m, 4H, 2 × CH$_2$), 2.58 (m, 10H, 5 × CH$_2$), 3.31 (s, 2H, CH$_2$), 5.87 (s, 2H, OCH$_2$O), 6.28 (brs, 1H, =CH), 6.44 (m, 2H, $J = 8$ Hz, Ar−H), 6.81 (m, 2H, $J = 8$ Hz, Ar−H); MS (EI) $m/z$ 316 (21.9). Oxalate salt: mp 167−69 ºC. Anal. [C$_{20}$H$_{32}$N$_2$O$_2$·2(CO$_2$H)$_2$] 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$_3$) $\delta$ 1.02 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.43 (m, 2H, CH$_2$), 1.65 (m, 2H, CH$_2$), 2.56 (m, 10H, 5 × CH$_2$), 3.26 (s, 2H, CH$_2$), 4.99 (s, 2H, CH$_2$Ph), 6.23 (brs, 1H, =CH), 7.11 (m, 9H, Ar−H); MS (EI) $m/z$ 378 (13.2). Oxalate salt: mp 141−42 °C. Anal. [C$_{25}$H$_{34}$N$_2$O·2(CO$_2$H)$_2$] C, H, N.

**Typical Procedure for 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 compound **4e** (2.0 g, 5.3 mmol) in 30 mL of dry ether. Small amount of sodium chips was then added at a temperature between −40 and −45 °C till the reaction mixture turned 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 °C) and then solid ammonium chloride was added till the solution became 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 g, 98%): IR (neat) 3380 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 0.98 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.53 (m, 4H, 2 × CH$_2$), 2.49 (m, 10H, 5 × 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$_2$O) ND.

**Typical Procedure for Preparation of Compound 6.** To a stirred solution of compound **5** (0.5 g, 1.73 mmol) in 30 mL of dry acetone were added baked potassium carbonate (0.4 g, 6.8 mmol) and *N,N*-diethylaminoethylpyrrolidine hydrochloride (0.35 g, 2 mmol). The reaction mixture was refluxed for 20 h; 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 3-[(4-(2-pyrrolidin-1-yl)methoxyphenyl)methylene]-1-(3-*N,N*-diethylaminopropyl)pyrrolidine as an oil (0.45 g, 68%): $^1$H NMR (CDCl$_3$) $\delta$ 1.02 (t, 6H, $J = 8$ Hz, 2 × CH$_3$), 1.78 (m, 8H, 4 × CH$_2$), 2.58-(m, 14H, 7 × 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, $J = 8$ Hz, Ar−H), 7.11 (d, 2H, $J = 8$ Hz, Ar−H); MS (EI) $m/z$ 385 (43.2). Oxalate salt: mp 118−21 °C. Anal. [C$_{25}$H$_{34}$N$_2$O·H$_2$O·3(CO$_2$H)$_2$] C, H, N.

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