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Fast atom bombardment mass spectral analysis of three new oxidative products of primaquine

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Abstract

FAB mass spectrum of 5,5-di-[6-methoxy-8-(4′-amino-1′butyl amino)] quinoline (PI) was recorded in three different concentrations to establish the structure of new oxidative products of primaquine and also the effect of matrix on percentage relative abundance of molecular as well as fragment ions which were demonstrated first time. At three concentrations, three different behaviors of matrix, molecular and fragment ions were observed. At higher concentration (>1 nM) molecular ion behaved as a base peak, but due to side reaction with matrix certain extra peak were also obtained. Thus, the higher concentration was interesting to show the side reaction of analyte with *m*-nitro-benzyl alcohol (NBA) but not for molecular weight determination. At the lowest concentration (<0.6 nM) matrix ion behaved as a base peak in addition to molecular ion. Therefore, this region of concentration is useful for determining the molecular weight of the compound. However, the mass spectra of 0.6–1 nM concentrations were useful for structural elucidation as compared to mass spectra below 0.6 nM and above 1 nM. Thus, fast atom bombardment mass spectrum with NBA as a matrix was recorded in between 0.6 and 1 nM concentrations for the structural elucidation of new compounds. Molecular ions and fragment ions of 5,5-di-[6-methoxy-8-(4′-amino-1′ butyl amino)] quinoline (PI), 6-methoxy-5,8-di-[4′amino-1′-methyl butyl amino] quinoline (PII), and 5,5-di-[7-hydroxy-6-methoxy-8(4′-amino-1′-methyl butyl amino)] quinoline (PIII) were identified. The fragment ions were obtained due to ring cleavages, Retero–Diels–Alder reaction (RDA), loss of side chain, proton transfer and substituted groups of the ring. On the basis of the fragmentation schemes and molecular ion peaks the structure of three new compounds PI, PII and PIII were proposed. In vitro studies showed that the compounds PI and PII had four times more gametocytocidal activity than primaquine but the compound 6-methoxy-5,8-di-[4′-amino-1′-methyl butyl amino] quinoline (PII) was found to have good gametocytocidal activity against *Plasmodium yoelli* infected mice at 10 mg kg^{-1} dose in vivo. Therefore, the spectra reported might serve as reference for the development of new molecules for the radical cure of relapsing malaria.

Keywords: FAB mass spectrometry; Matrix effect; Anti-malarial drug; In vitro; In vivo

1. Introduction

Primaquine, an 8-aminoquinoline is the clinical drug of choice for the radical cure [1] of relapsing malaria. However, its usefulness has been restricted due to its toxic side effects especially in patients with deficient glucose-6-phosphate dehydrogenase [2]. Therefore, there is always a recognized need for investigation of less toxic and more effective new tissue schizontocide. Several analogous of primaquine were synthesized by chemical [3–6] and oxidation [7] methods and screened for anti-malarial activity but none was found better than primaquine. Earlier we have isolated three compounds in which two compounds have shown more gametocytocidal activity than primaquine [8]. The structure of two new oxidative primaquine analogous 5,8-di-[4′-amino-1′-methyl butyl amino]-6,7-dihydroxyquinoline and *n*,*n*-tri-(4′-amino-1′-methylbutylamine) were identified by me recently using EI–MS [9]. Idowu et al. [10] have used LC–MS and GC–MS for the characterization of metabolites of WR238605, an 8-aminoquinoline by rat liver microsomes and reported electron impact mass spectrum of these derivatives. Field desorption mass spectral analysis of dimmer of *n*-acetylated primaquine formed by microbial transformation of primaquine by *Candida tropicals* was reported earlier [11]. Strother et al. [12] have used

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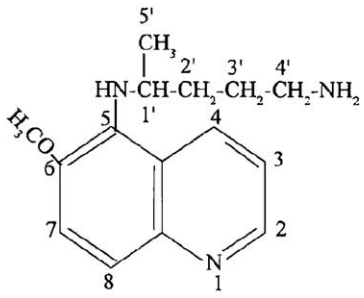
mass spectrometry in electron impact (EI) and chemical ionization mode for the identification of blue derivative of primaquine metabolite, which forms during its in vitro metabolism by hepatic enzymes of mice. Hufford et al. [13] have used field desorption mass spectra for the characterization of sulfer linked dimmer of *n*-acetyle primaquine. It was isolated by microbial metabolism of primaquine by *Streptomyces roseochromogenus*. Fast atom bombardment (FAB) mass spectrometry was used for the elucidation of structures of thermally labile compounds of high molecular weight [14–20] as the molecular ion peak of higher molecular weight cannot be obtained by EI mass spectrum. In this paper the FAB mass spectral analysis of three new primaquine analogous are reported first time and also the correlation between the mass spectral data and its structural features are discussed.

2. Experimental

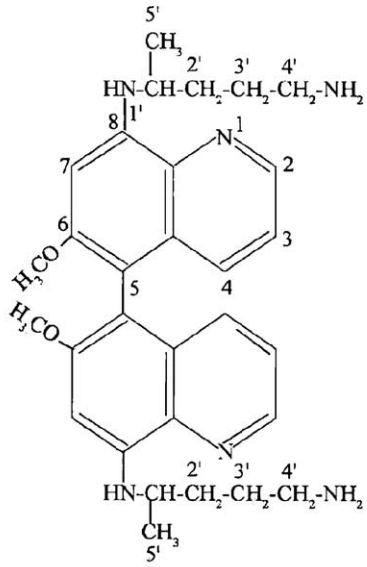
Earlier, we have isolated three oxidative products of primaquine [8]. For isolation of these compounds the 2 ml aqueous solution of 2.5 mg/ml primaquine and 2.4 mg/ml $K_2S_2O_8$ each were mixed together. After 20 min of initiation, the reaction mixture was loaded and fractionated on bio-gel P-2 (<45 mesh) column for preparative

Table 1
Structure of some products of primaquine



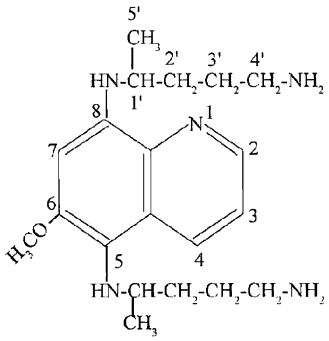
Mwt = 259

Primaquine



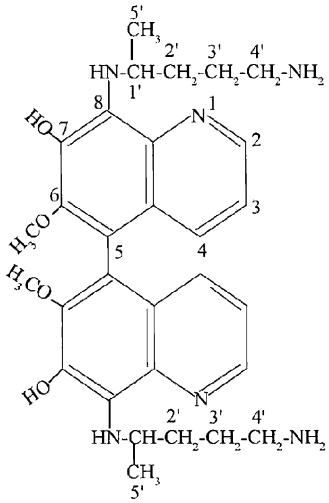
Mwt = 516

5,5 di-[6-methoxy, 8(4'-amino-1'-methylbutylamino)] quinoline [P(I)]



Mwt = 359

6-methoxy-5,8 di-(4'-amino-1'-methylbutylamino)-quinoline [PII]



Mwt = 548

5,5 di-[7-hydroxy-6-methoxy, 8(4'-amino-1'-methyl butyl)amino] quinoline [P(III)]

isolation of different oxidation products using water for elution. Purity of each fraction was tested using HPLC method. The HPLC apparatus consisted of a Waters 510 pump, a Rheodyne 7125 injector, variable wavelength UV 486 detector operated at 254 nm and an integrator. The mobile phase acetonitrile–methanol–perchloric acid (1 M)–water (30:7:1:95, v/v) was pumped at a flow rate of 1.0 ml/min through μ Bondapak C_{18} reverse phase column (300 mm × 3.9 mm; particle size, 5 μm) and the structures are given in Table 1. The chemical structures of these compounds were established using UV, IR, high field and ^{1}H ^{13}C NMR spectral data and also screened for anti-malarial activity in our earlier studies [21,22].

The EI spectrum was recorded on JEOL JMSD-300 spectrometer at 70 eV using direct inlet system.

The FAB mass spectra were recorded on a JEOL SX-102/DA-6000 mass spectrometer/data system using a 6 keV xenon beam (10 mA) equipped with the FAB target (a metal probe tip 3–4 nm^2 made of stainless steel). *m*-Nitro benzyl alcohol (NBA) was used as matrix. One microliter of matrix was deposited on the FAB target and concentration of 0.5–1.8 nM of sample solutions in distilled water was added. The spectra were recorded in three different concentrations (>0.6 nM, in between 0.6 and 1 nM and <1 nM) to demonstrate the effect of matrix on relative abundance of molecular ion as well as fragment ions and also to establish the structure of new compounds. The target was subjected to FAB mass in such a way, that atom flux strike the sample and spread on the FAB target at 70° angle of incidence. In the scaddle field source electrons were induced to oscillate between two cathodes under the action of a dc field. The FAB desorbed ions were accelerated to 10 kV at 10^{-4} Torr pressure and could produce a flux of ions and neutrals upto the equivalent of a 500 μA charged beam.

The parameters of the experiments are given below.

Analyte	Ions derived (for base peak) (*m*/*z*)	Retention time (s)	Intensity	Scan*
PI	154[a]	0.36″	93.4016	6–8
PII	175[b]	0.24″	70.087	1–4
PIII	55[c]	0.12″	78.2918	1–3

*Scan of FAB mass spectra of analyte were recorded in average scan of 1–3, 1–4, 6–8. (a) For matrix ion peak. (b and c) For fragment ion peak.

3. Result and discussion

Primaquine, on oxidation with peroxydisulphate ion in neutral medium gave pale yellow, orange, brown, violet and then yellow colour within 1 h of initiation of reaction. The

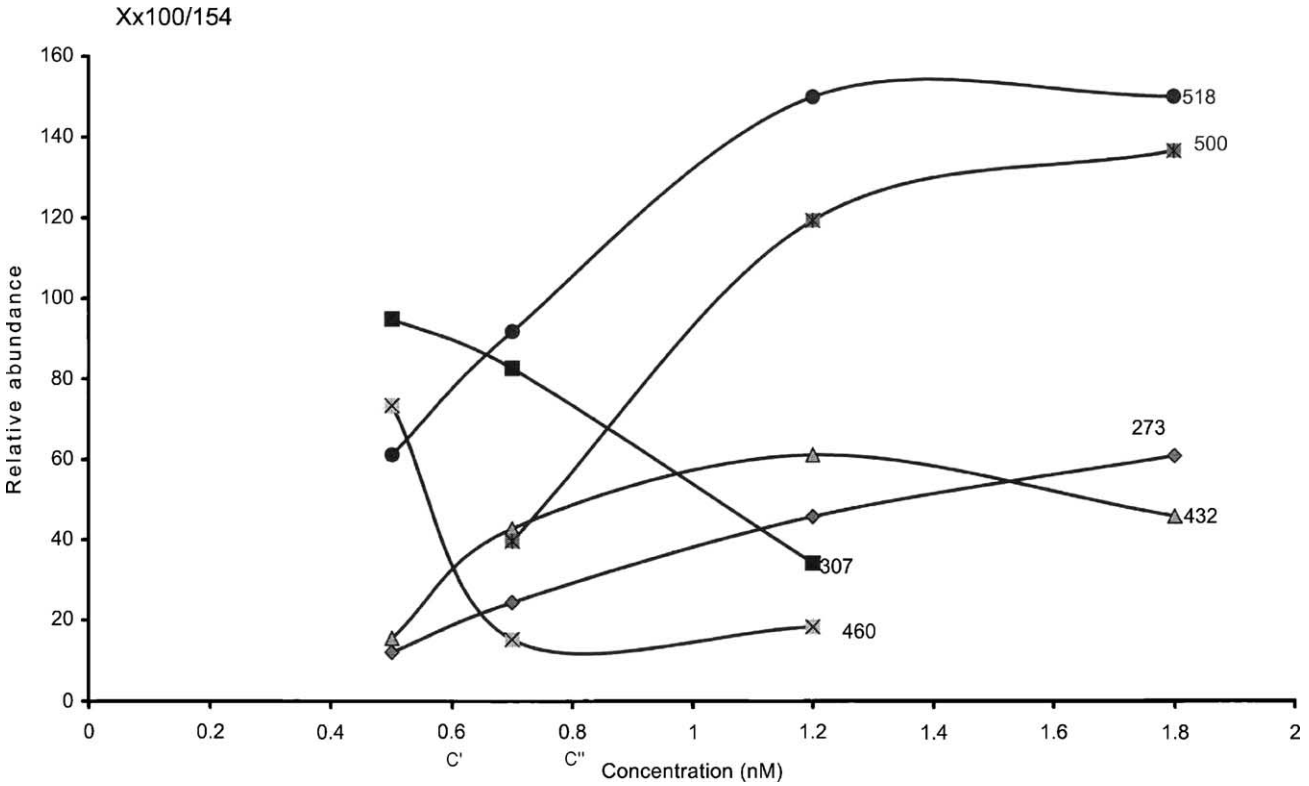
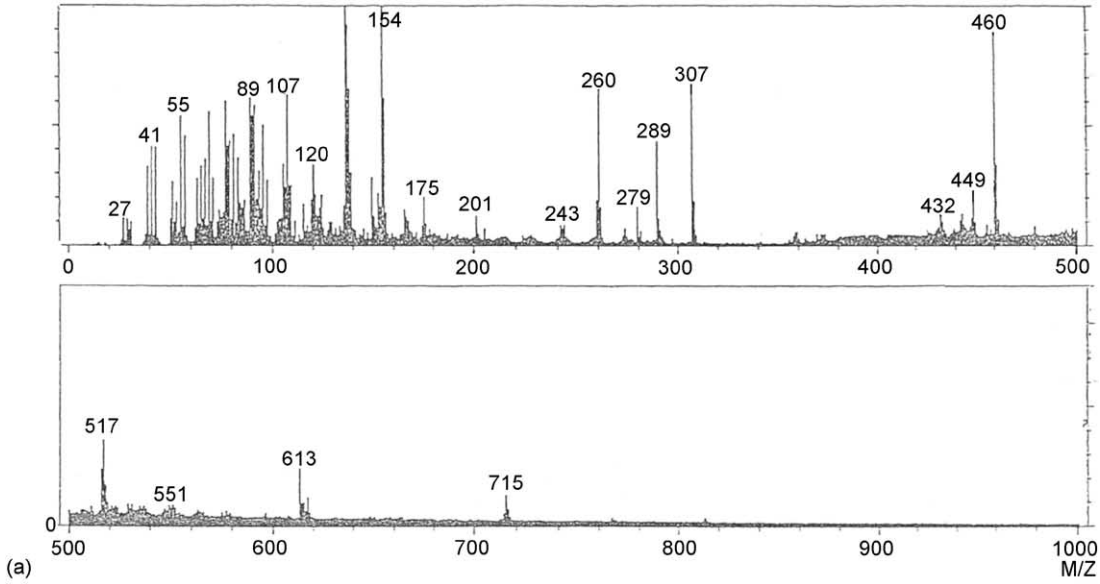
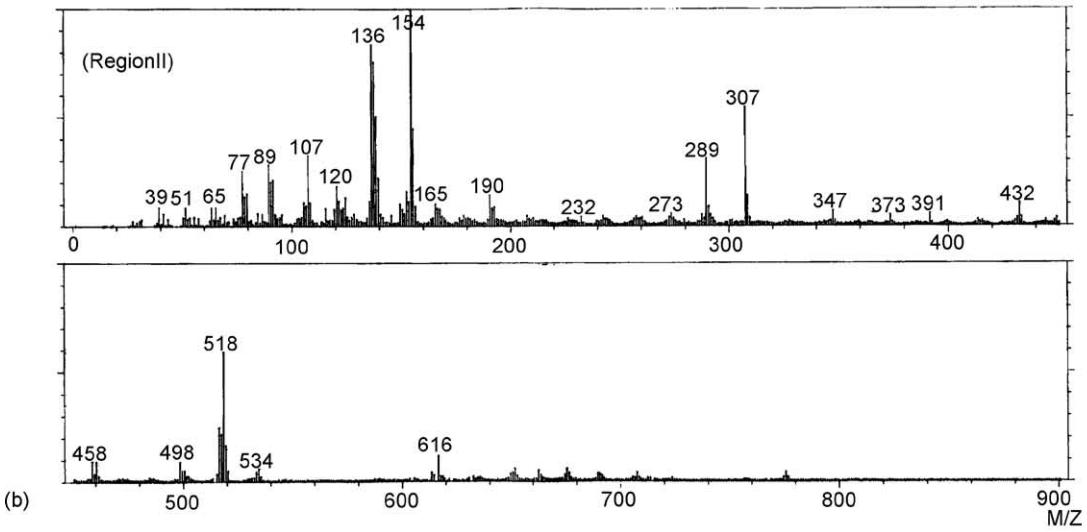


Fig. 1. Effect of the amount of oxidation product 5,5-di-[6-methoxy-8-(4′-amino-1′-methyl butyl amino)] quinoline (PI) on ion formation in 1 μl of *m*-NBA matrix in the positive ion FAB mass spectrometry on concentration 0.5–1.8 nM of oxidative product. The amount of PI is expressed in nanomole. The peak intensities are related to the peak at *m*/*z* 154 of the NBA matrix. The peak at *m*/*z* 518 represents pseudomolecular ion while the *m*/*z* at 500, 432, 373 was observed for fragement ion and *m*/*z* at 154, 307 and 460 for matrix ion.





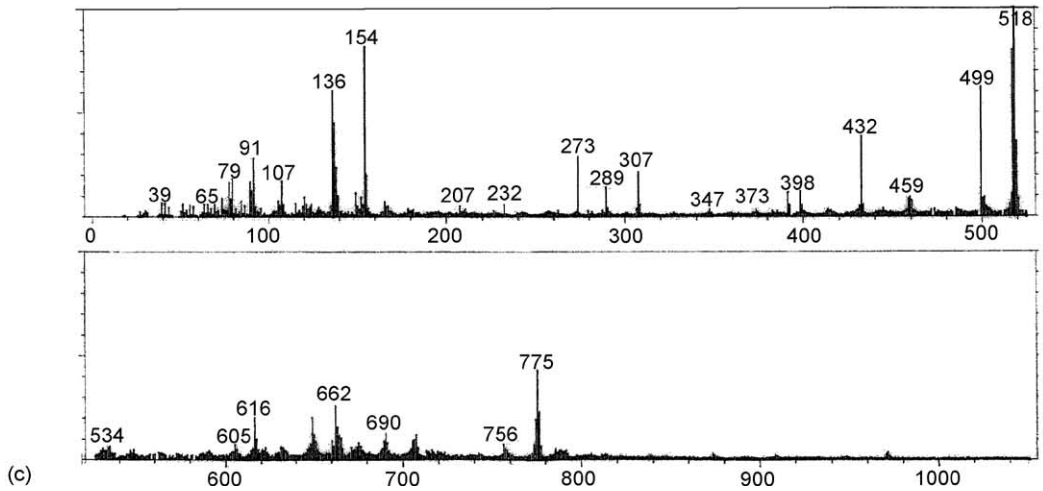


Fig. 2. FAB mass spectra recorded for compound PI (a) below 0.6 nM; (b) in between 0.6–1 nM; (c) above 1 nM concentration.

UV spectra recorded at different times gave different λ_{max} indicating formation of different compounds during the course of the reaction. Different colour bands were obtained on bio-gel P-2 column and different fractions were collected manually. Each fraction was further tested for their purity (>95%) using high-performance liquid chromatography [7]. The structure of these compounds, 5,5-di-[6-methoxy-8-(4′-amino-1′-butyl amino)] quinoline (PI), 6-methoxy-5,8-di-[4′-amino-1′-methyl butyl amino] quinoline (PII), and 5,5-di-[7-hydroxy-6-methoxy-8(4′-amino-1′-methyl butyl amino)] quinoline (PIII) were chemically established by spectral analysis like IR and NMR earlier [21,22]. In vitro studies showed that the compound PI and PII had three or four times higher gametocytocidal activity than primaquine, while PIII had lower activity. IC 50 and IC 90 of compound PI were 0.039 and 0.62 μg per well, while PII were 0.026 and 0.055 μg per well, respectively. No significant schizontocytocidal effect was observed with respect to chloroquine [8]. Our earlier study [22] showed that the compound PII has good gametocytocidal activity against *Plasmodium yolli* infected mice at 10 mg kg^{-1} dose in vivo.

The *m*-NBA cultures ions are found of masses corresponding to multiples of molecular weights of *m*-NBA plus a proton; *m*/*z* 136, 154, 289, 307, 460, 613 and the bombardment of analytes/*m*-NBA mixture results in sputtering into the gas phase of $[M + H]^+$and $[M - H]^-$ ions of the analytes [23,24]. Such ions may exist in the analyte/matrix solution due to proton transfer process and also protonated molecular ion fragments were obtained due to loss of neutral molecules [25,26]. A protonated alcoholic hydroxyl group is likely to be lost as water via at the charge site initiated cleavages with charge transfer. In addition to charge-site initiated cleavages, proton transfer and charge retention were also observed [27,28].

Primaquine was taken as standard reference compound for the interpretation of MS spectral data. The different behaviors of compound PI in different concentration are shown in Fig. 1.

These results reveal three regions in the FAB mass spectrum of compound PI (Fig. 1) in relation to its concentration in matrix. In region I concentration lower than C′ (<0.6 nM), a protonated molecular ion $[M + H]^+$ and a series of matrix derived ion (high percentage relative abundance) are produced. Region II, in the range between C′ and C″ (0.6–1) still shows the protonated ion $[M + H]^+$ but also has several fragment ions at higher concentration. In region III, the concentration of compound PI is greater than C″(>1), the protonated pseudomolecular ion at *m*/*z* 518 as a base peak and matrix ion peak was also observed at lower percentage relative abundance.

The behavior of the compound PI is illustrated in Fig. 2a–c. In region I (Fig. 2a) a pseudomolecular ion at *m*/*z* 517 $[M + H]^+$ was observed. But in this region the high intensity matrix ions at *m*/*z* 154 (as base peak), 136, 289, 307,460 were also obtained. In region II (Fig. 2b) the intensity of the matrix ions decreases and the protonated molecular ion with fragment ion (500, 432, 391, 373, 347, 273) increases. In region III (Fig. 2c) the peaks of matrix ion decrease and the pseudomolecular protonated ion peak at *m*/*z* 518 behaves as a base peak. In this region besides the fragment molecular ion several extra peaks were observed at *m*/*z* 662 and 775 might be due to side reaction with NBA matrix with analyte leads to form a bigger molecule $C_{30}H_{40}N_6O_2$ is demonstrated in Fig. 9. The peak at *m*/*z* 518 (M + 2H) was observed in this region due to the reduction of the analyte with matrix. Similar type of observation was earlier reported [14] and suggested that the most common chemical reactions inferences of matrix reported is the

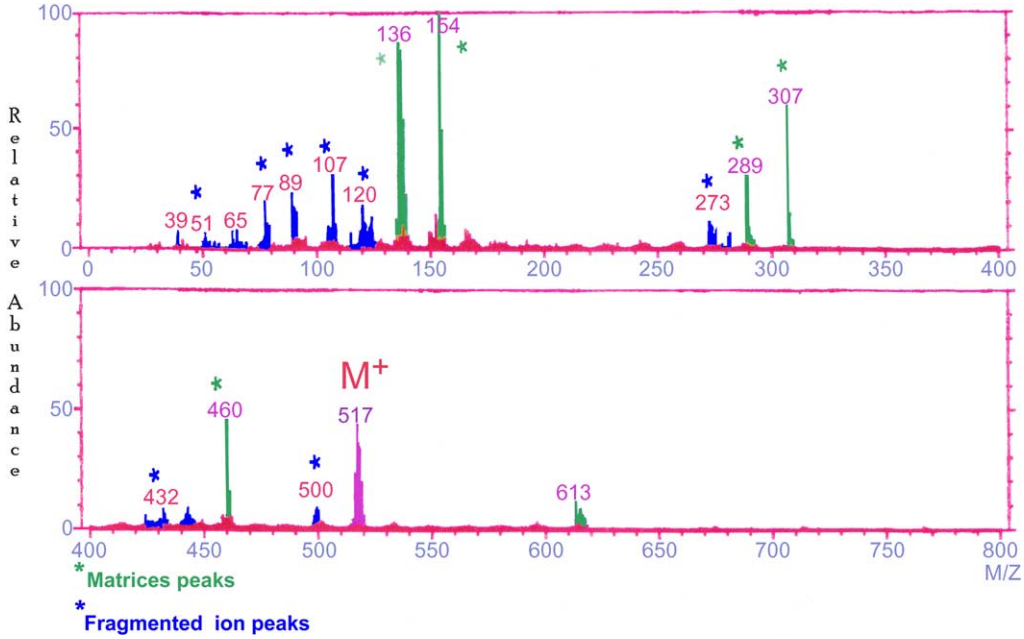


Fig. 3. FAB mass spectrum of 5,5-di-[6-methoxy-8-(4′-amino-1′-methyl butyl amino)] quinoline (PI).

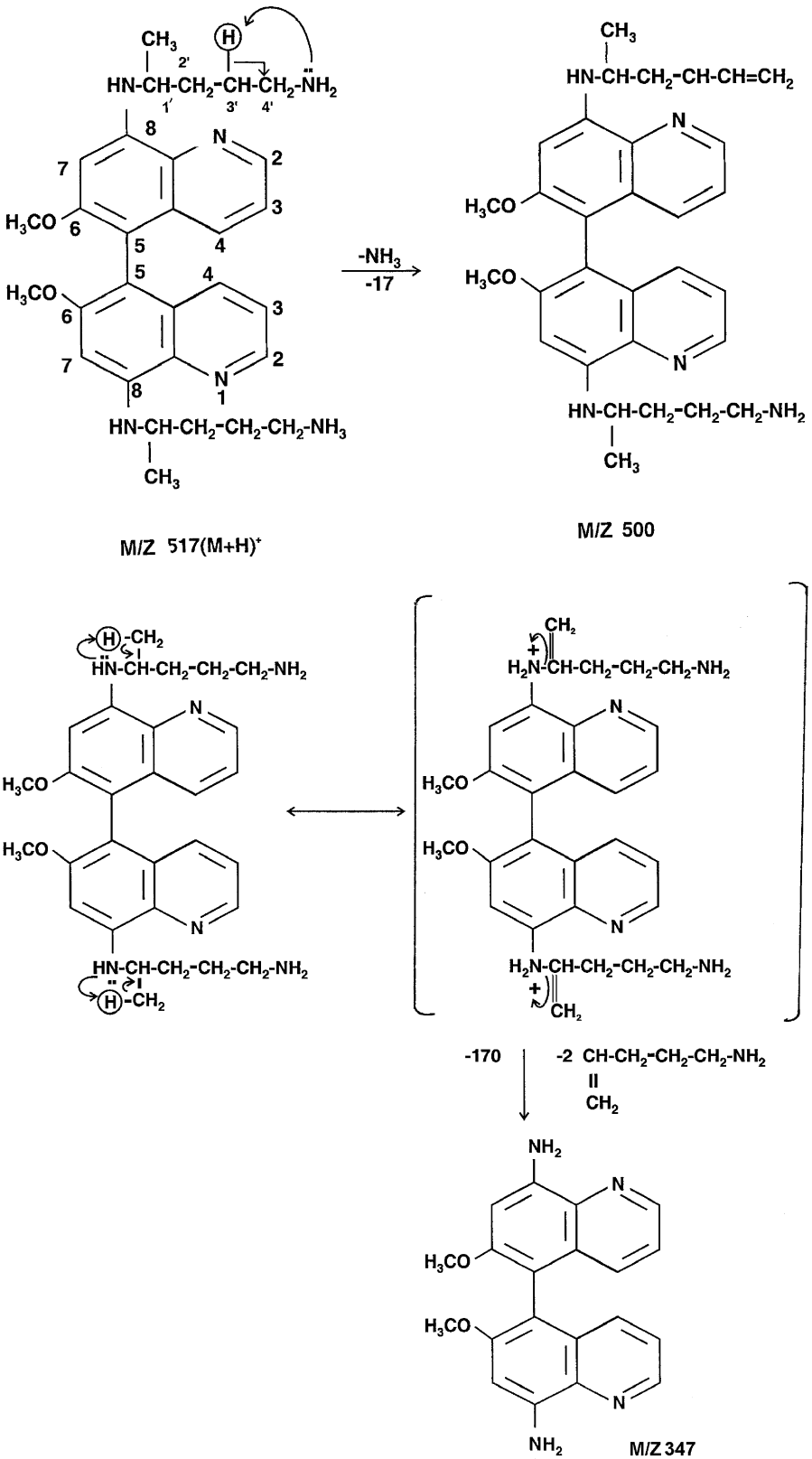


Fig. 4. Illustration of the fragmentation scheme in FAB mass spectrum of 5,5-di-[6-methoxy-8-(4′-amino-1′-methyl butyl amino)] quinoline (PI).

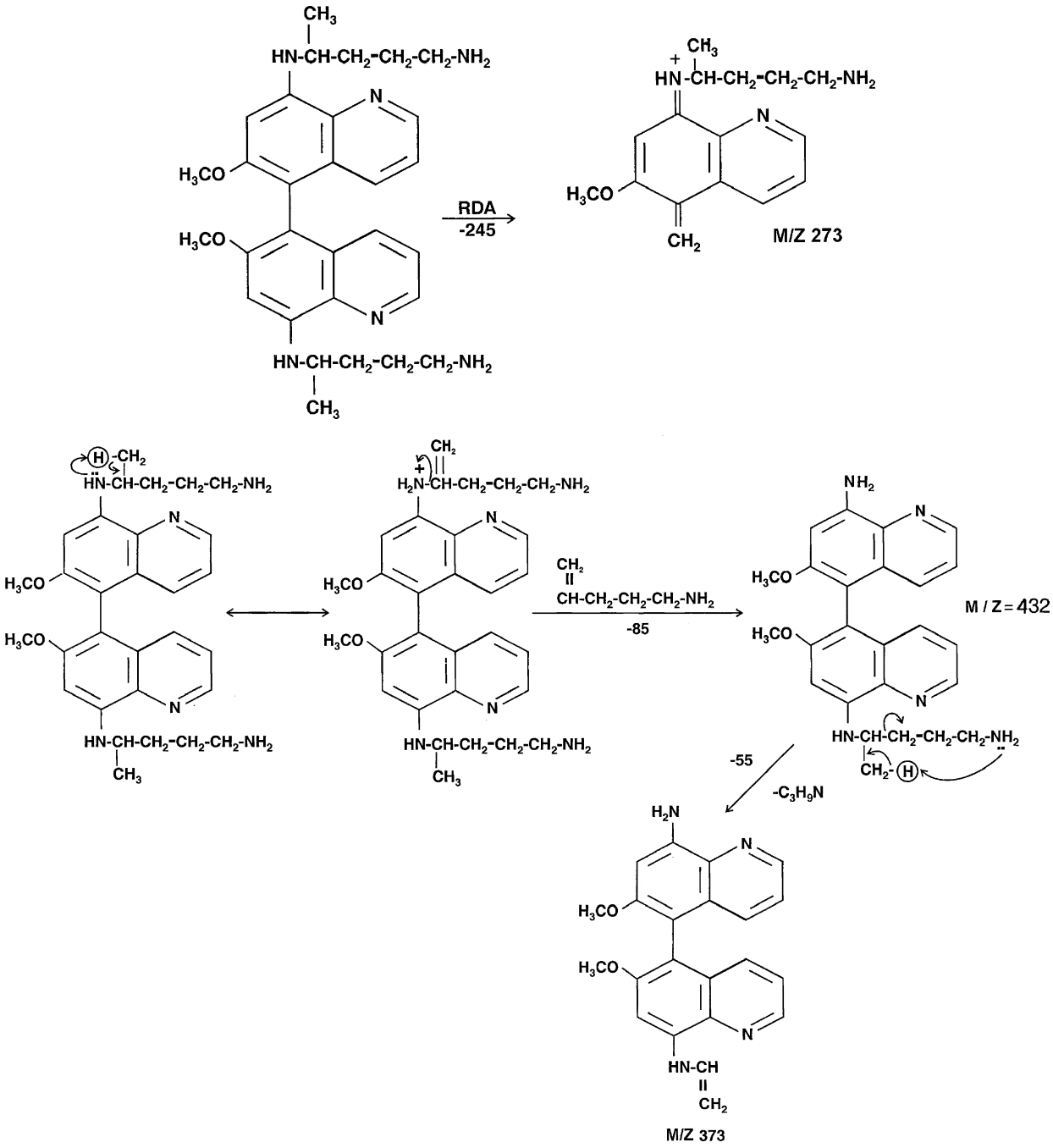


Fig. 4. (*Continued*).

reduction of the analyte to form (M + 2H) and (M + 3H) ions.

We conclude that the region I is suitable for determining the molecular weight of compound while the region II is very useful for structural illustration and III region was interesting to show the side reaction of analyte with NBA. In the region III the extra peak like *m/z* 775 and 662 arising from chemical reaction occurring between the matrix and the analyzed sample and also may be due to the impurities present in trace amounts. Therefore, this region is not suitable for determining unambiguously the molecular weight of the analyzed sample. Our finding is supported by earlier reported study [23]. Therefore, the region II was chosen for structural interpretation of compound.

3.1. Structural elucidation of 5,5-di-[6-methoxy-8(4′-amino-1′-methyl butyl amino)] quinoline (PI)

EI mass spectrum failed to give molecular ion peak. Therefore FAB mass spectrum was used for structure elucidation (Fig. 3). The intense peak at *m/z* 517 corresponds to the protonated pseudomolecular ion peak $[MH]^+$. The

assignment of molecular ion at *m*/*z* 517 was also consistent with nitrogen rule. The weak intensity peak at *m*/*z* 500 was due to loss of ammonia gas from $[MH]^+$, which confirms the presence of terminal amino group at 4′ position. This type of fragmentation scheme was observed during the fragmentation of another new oxidative primaquine analogous 5,8-di-[4′-amino-1′-methyl butyl amino]-6,7-dihydroxyquinoline by me in earlier study [9]. The fragment ion peak at *m*/*z* 347 was assigned due to loss of two molecules of butyl-amine $C_{10}N_2H_{23}$ (17O μ) from the molecular ion $[MH]^+$. Generally in the FAB cases the different fragment ions occur due to loss of neutral molecule [26] from the molecular ion (M + 1) and present results are in agreement with the stabilized phenomenon. At *m*/*z* 432 was assigned due to loss of CH_3–CH=CH–CH_2–CH_2–NH_2 (85 μ) group from the molecular ion $[MH]^+$. The ejection of propyl-amine CH_3–CH_2–CH_2–NH_2 (59 μ) from the fragment ion [MH] − 85 affords an ion at *m*/*z* 373. This fragmentation schemes is in the line with the exemplary study carried out by Das et al. [21] and Sinha [9]. The *m*/*z* value at 272 was due to the loss of one quinoline group at the 5 positions i.e., ($C_{14}N_3H_{18}O$) from the molecular ion $[MH]^+$ and also having an isolated double bond in a six-member ring. This type of cleavage in ring was observed due to the result of charge remote Retro–Diels–Alder reaction (RDA). Similar type of finding was observed in case of stable terpenoid and steroidal compound reported earlier [28–31]. All the above peaks confirmed the presence of extra primaquine unit at C-5 position (dimmer of primaquine) with molecular formula $C_{30}H_{40}N_6O_2$ (molecular weight 516 μ). The fragmentation schemes (Fig. 4) confirm the structure of PI.

3.2. Structural elucidation of 6-methoxy-5,8-di-[4′-amino-1′-methyl butyl amino] quinoline (PII)

The intense peak at *m*/*z* 358 was assigned to deprotonated quasimolecular ion $(M - H)^-$ peak (Fig. 5a). It

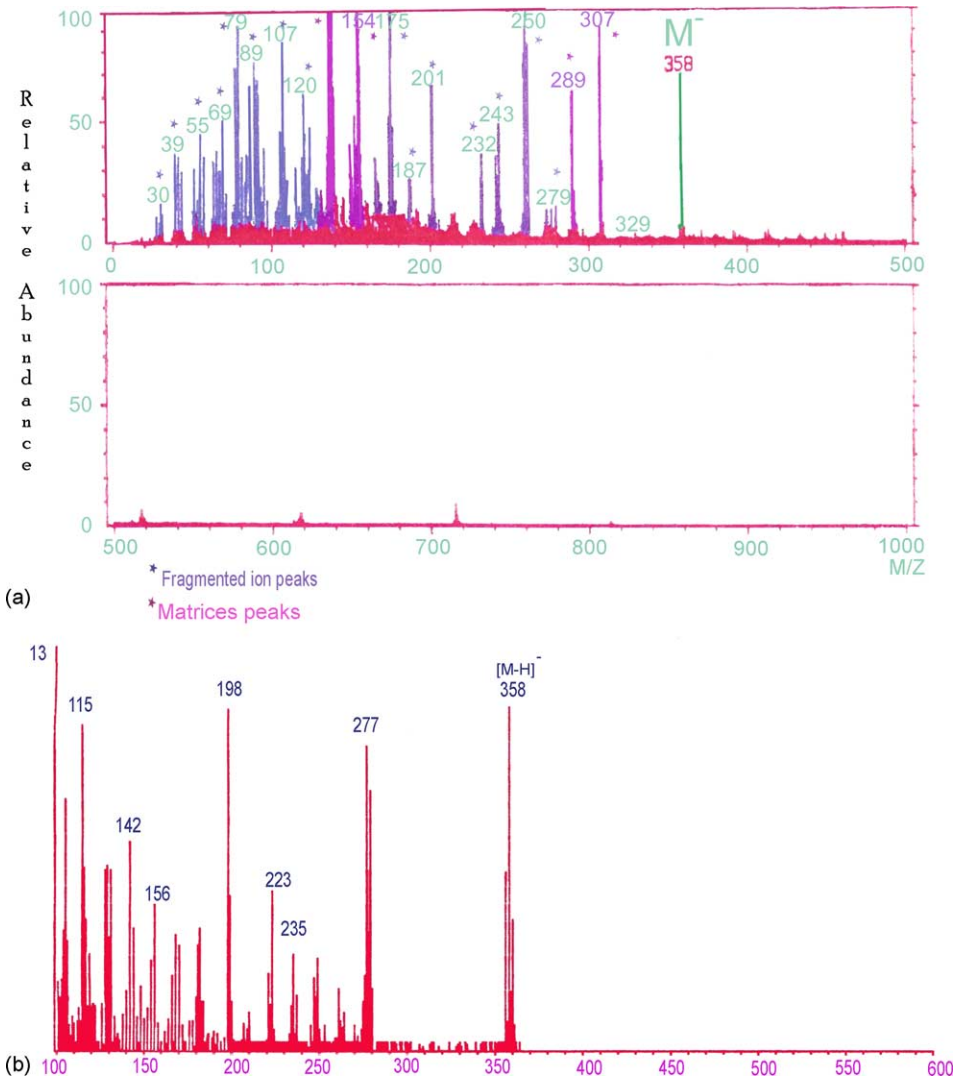


Fig. 5. (a) FAB mass spectrum of 6-methoxy-5,8-di-[4′-amino-1′-methyl butyl amino] quinoline (PII). (b) Electron ionization mass spectrum of 6-methoxy-5,8-di-[4′-amino-1′-methyl butyl amino] quinoline (PII).

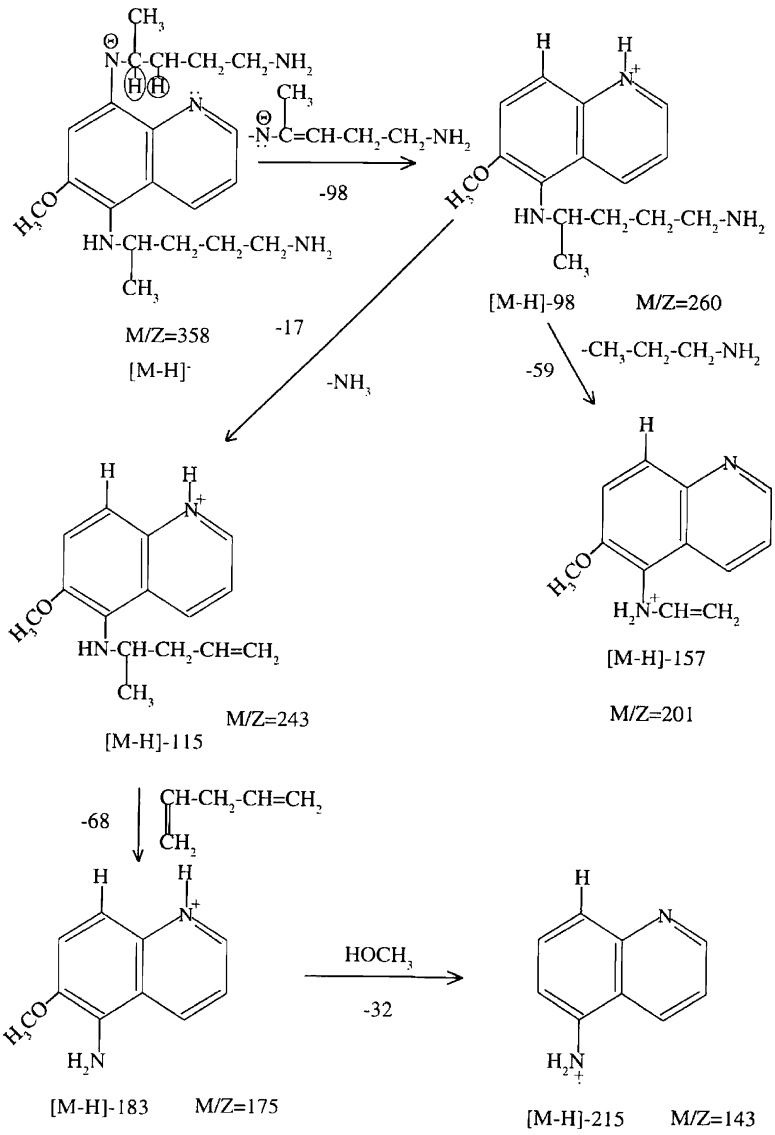


Fig. 6. Illustration of the fragmentation scheme in FAB mass spectrum of 6-methoxy-5,8-di-[4′-amino-1′-methyl butyl amino] quinoline (PII).

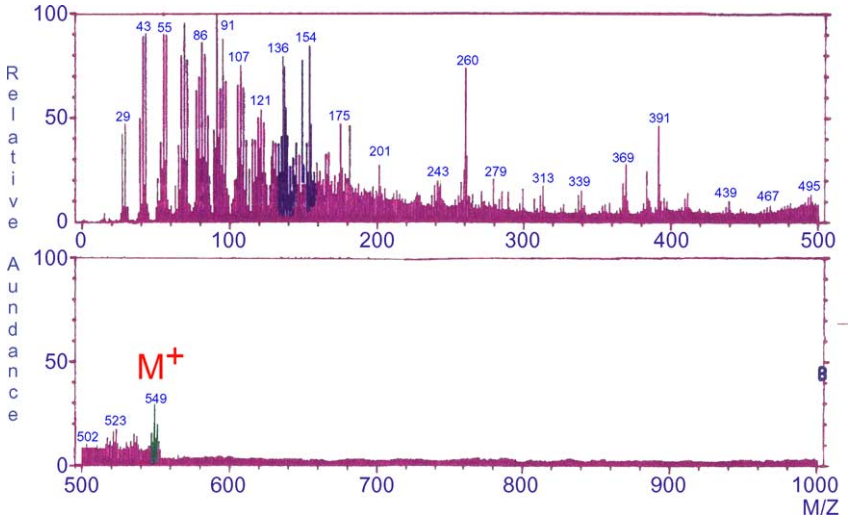


Fig. 7. FAB mass spectrum of 5,5-di-[7-hydroxy 6-methoxy-8-(4′-amino-1′-methyl butyl amino)] quinoline (PIII).

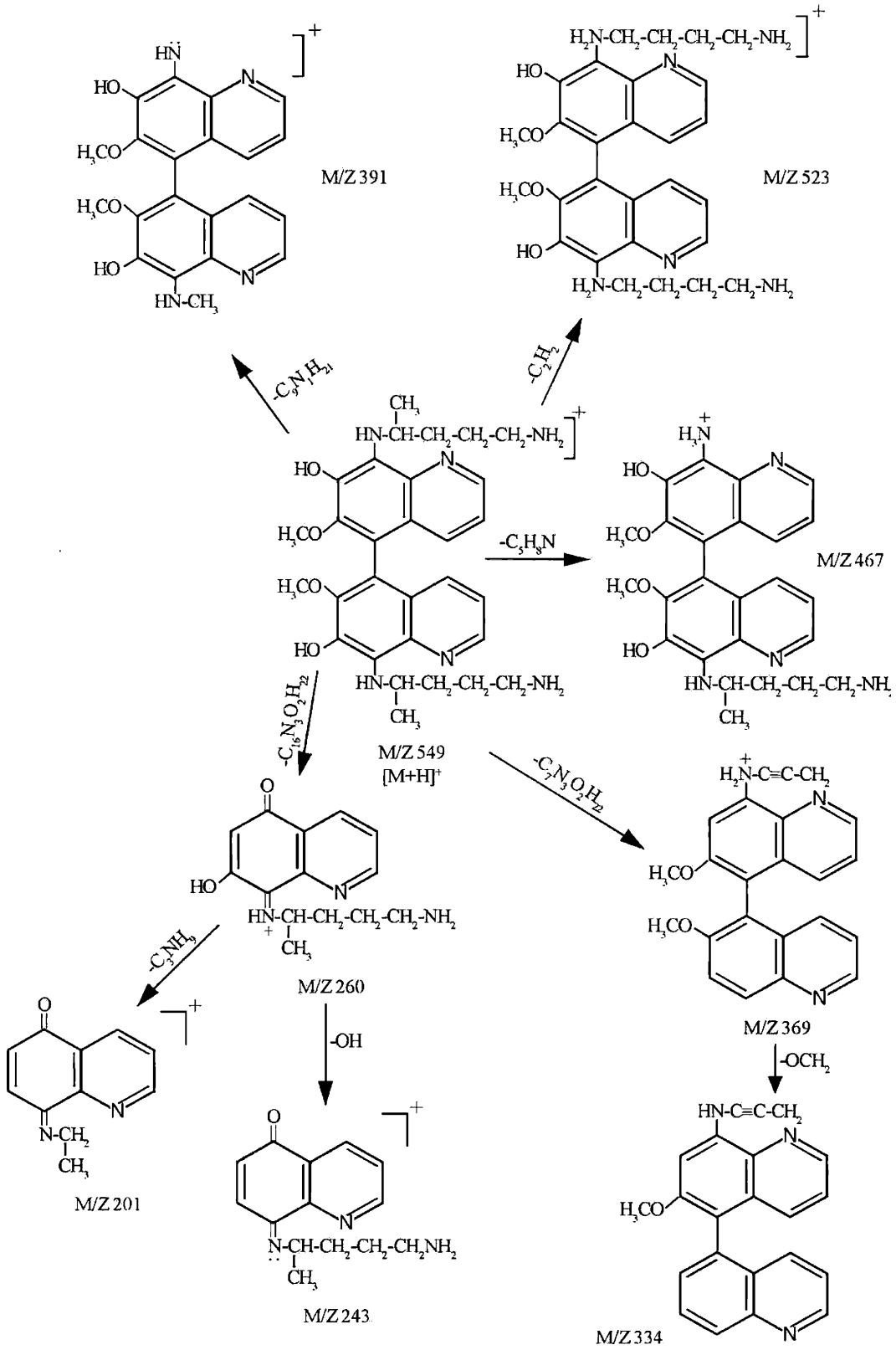


Fig. 8. Illustration of the fragmentation scheme in FAB mass spectrum of 5,5-di-[7-hydroxy 6-methoxy-8-(4′-amino-1′-methyl butyl amino)] quinoline (PIII).

may be pointed out that EI mass spectra also gave intense quasimolecular ion $(M - H)^-$ peak at *m*/*z* 358 (Fig. 5b). The peak at *m*/*z* 260 was due to the loss of –N–C (CH_3)=CH–CH_2–CH_2–NH_2(98 μ) group from the $(M - H)^-$ molecular ion and this fragment clearly showed that in primaquine an extra side chain (4′-amino-1′-methyl-*n*-butylamine) was added with intact all other groups. The peak at *m*/*z* 243 could be obtained due to the loss of ammonia gas $–NH_3$ (17 μ) from fragment ion $[(M - H)^- - 98]$ which confirms the presence of terminal amino group. Loss of (propylamine) $–CH_3–CH_2–CH_2–NH_2$ (59 μ) from the $[(M - H)^- - 98]$ ion affords a peak at *m*/*z* 201. At 175 was due to the loss of $–CH_2$=CH–CH_2–CH=CH_2 (68 μ) group from fragment ion $[(M - H^-) - 115]$ and *m*/*z* 143 was due to expulsion of methyl alcohol ($–HOCH_3$) (32 μ) from fragment ion $[(M - H)^- - 215]$. All the above peaks confirmed the presence of an extra aliphatic side chain i.e., –NH–CH (CH_3)–CH_2–CH_2–CH_2–NH_2 in primaquine. We are reporting first time the fragmentation scheme of this new compound using FAB, though in similar line FAB fragmentation is reported for phenoxazine, an anticancer drug [32].

The mass spectral studies of the component PII indicate the presence of two aliphatic side chain with molecular formula $C_{20}H_{33}N_5O_1$ (molecular weight 359 μ) and

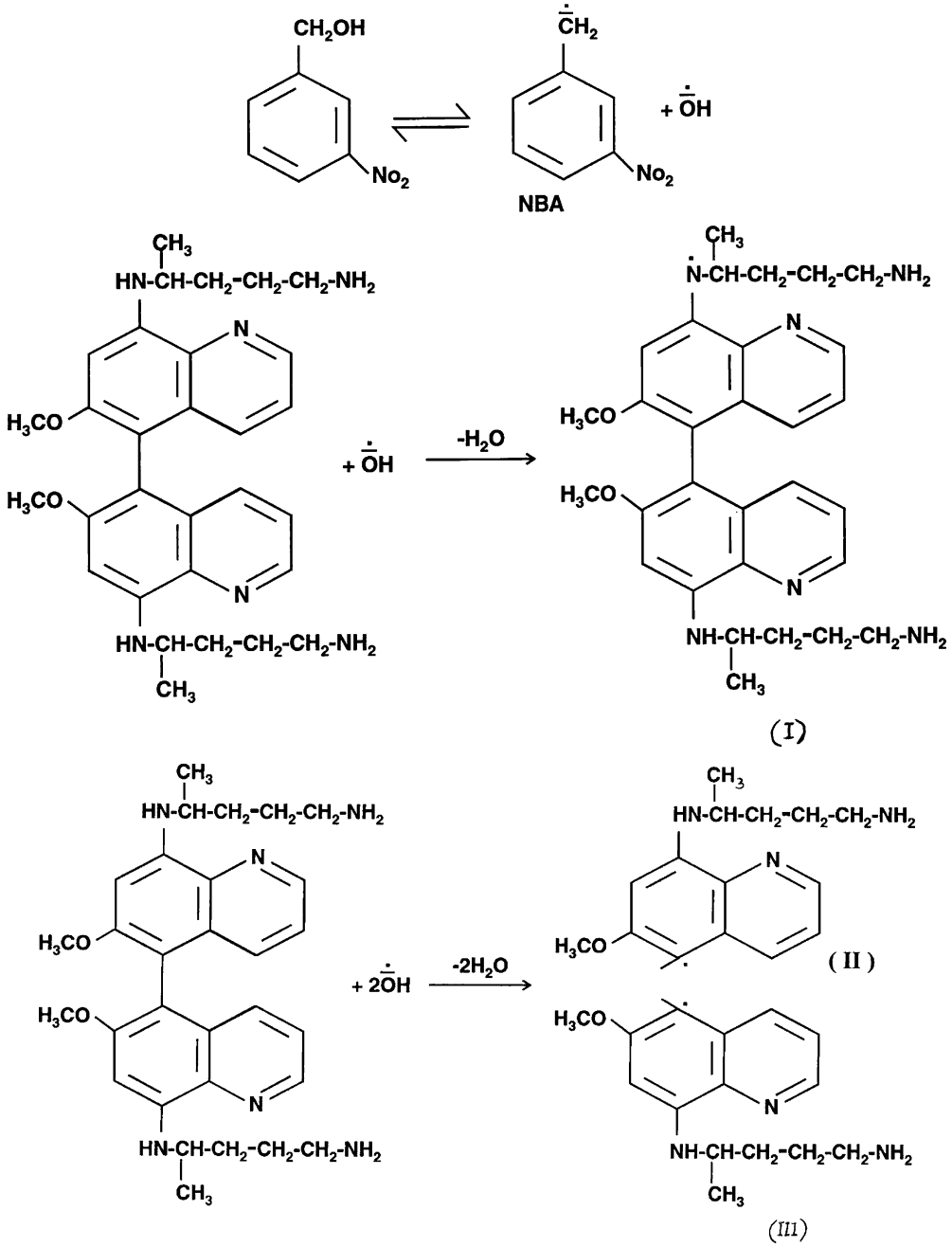


Fig. 9. Illustrations of side reaction of analyte PI with matrix.

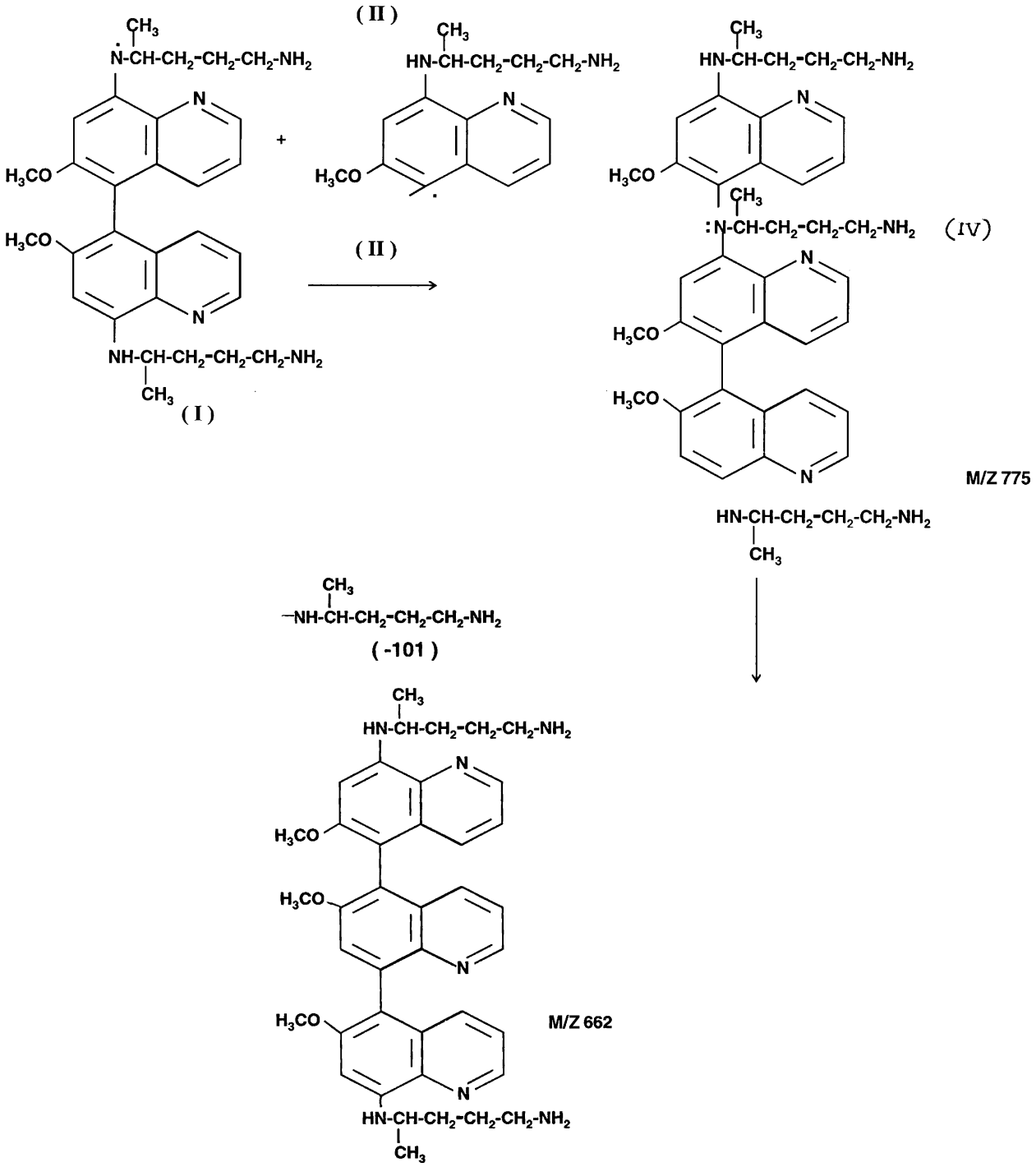


Fig. 9. (*Continued*).

the fragmentation scheme (Fig. 6), confirms the proposed structure of PII.

3.3. Structural elucidation of 5,5-di-[7-hydroxy-6-methoxy-8(4′-amino-1′-methyl butyl) amino] quinoline (PIII)

The FAB mass spectrum of compound (PIII) is shown in Fig. 7. The low intensity peak at *m/z* 549 was assigned as protonated molecular ion peak $(M + H)^+$ implying that the molecular weight of the compound is 548 μ. *m/z* 523 was due to loss of (–CH=CH–) 26 μ from the protonated molecular ion $(M + H)^+$. The peak at 467 was due to expulsion of side chain as 1,3 butadiene amine CH_3–CH=CH–CH=CH–NH_2 (−82 μ) while *m/z* at 391 was due to loss of both side chain $–C_9H_{21}N$ (158 μ) from $[MH]^+$, respectively. The elimination of $–C_9H_{21}N$ (158 μ) from $[MH]^+$ results as *m/z* 369 confirmed the presence of

two side chain linkage (4′-amino-1′-methyl *n*-butyl amine) in protonated molecule, while the ejection of formaldehyde $–OCH_2$ (30 μ) at C-6 position from the fragment ion $[MH]^+ - 180$ affords an ion at *m*/*z* 339. *m*/*z* 279 was assigned due to loss of $H_2N–CH_2–CH_2–NH_2$ from $[MH]^+ - 210$. On the other hand, the rearrangement process of methyl radical results loss of 7-hydroxy-6-methoxy-5-methyl primaquine $–C_{16}H_{22}N_3O_2$ (288 μ) giving rise base peak at *m*/*z* 260, which is the characteristic ion in the protonated molecule $[MH]^+$. *m*/*z* at 243 and 201 were due to loss of H_2O and propyl amine $–CH_3–CH_2–CH_2–NH_2$ from the fragment ion $[MH]^+ - 288$. This confirmed the presence of –OH group in compound PIII. All the above peaks confirmed the presence of 7-hydroxy-6-methoxy primaquine at C-5 position. The fragmentation schemes of PIII (Fig. 8), confirmed the proposed structure.

4. Conclusion

FAB mass spectrometry was found very useful to assign the molecular ion peaks and fragmentation schemes for elucidation of structures in the present series of compounds of biomedical interest because EI MS failed to show molecular ion peak. Thus molecular weight and structures of these compounds were determined unambiguously using FAB mass spectrometry. The molecular ion peak at *m*/*z* 358 represented an increase of 100 unit mass as compared to primaquine (molecular weight 259 μ) which may be due to an addition of 4′-amino1′-methyl-*n*-butyl amine (side chain of primaquine) assigned the structure of PII. The fragmentation schemes and molecular ion peak revealed that the PI is a dimmer of primaquine linked at C-5 position intact the terminal amino groups. *m*/*z* 549 showed an increase of 32 μ mass as compared to PI (*m*/*z* 517). This is due to an addition of 2 –OH groups in PIII. The mass spectra reported here may serve as reference for this new series of compounds that may lead to new template molecules for the radical cure of relapsing malaria.

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